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THE NATURE AND SIGNIFICANCE OF THE STRUCTURAL CHANGES IN THE LUNGS IN MITRAL STENOSIS*

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INTRODUCTION

The lesions in cardiac disease are usually located in the left side of the heart, and it is the dysfunction of this side that is responsible for the majority of instances of circulatory failure. Ever since Corvisart's and Hope's contributions, morphologists have advocated a somewhat simplified mechanical "back pressure" concept of congestive failure. From time to time other explanations of circulatory failure have been offered, but the "back pressure" theory has received adequate support through the more recent physiological and chemical investigations. These studies have revealed that it is the disturbance of the pulmonary circulation that is the center of the problem of congestive failure.[1, 2] It is to the physiological and morphological changes within this circuit that the majority of the clinical manifestations are referable. While the study of the human pulmonary circulation during life has only recently become feasible, the effect of persistent passive congestion on the structure of the lungs has long been recognized and known under the term "brown induration." Furthermore, the pathological changes in the larger pulmonary arteries are familiar and are routinely looked for at autopsy, particularly in cases of mitral stenosis.

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Nevertheless, studies of the minute histological changes in the smaller blood vessels and alveolar capillaries have not been numerous. A detailed analysis of the relation of the morphological changes in the minute pulmonary vessels to structural alterations in the alveolar wall is also lacking. Thus it seems to us timely to analyze the physiological significance of the morphological alterations within the lungs in the presence of heart disease.

In a recent article zu Jeddeloh [3] has presented a review of the past studies of pulmonary congestion and in addition has reported his own observations on changes in the alveolar walls in chronic passive congestion. He did not include a study of the vascular changes apart from those of the capillaries. Brenner [4] has reviewed the literature on the pathology of pulmonary vessels; hence the presentation of such data is superfluous.

Our interest in this subject was aroused by the rather unusual clinical behavior and striking postmortem findings in a case of mitral stenosis, which is reported below. The study was later extended to a large group of cases presenting various types of pulmonary parenchymatous and vascular disturbances.

Material and Methods

In addition to the case reported in detail below, we have studied 9 cases of mitral stenosis. We have also examined the lungs in cases of rheumatic heart disease of varied pathology, of arterial hypertension, congenital heart disease, cardiac asthma, bacterial endocarditis, and in 1 case of marked kyphosis with right-sided hypertrophy and heart failure.

In the material under study during the past 2 years sections were taken from the upper and lower parts of each lobe and were run through separately. The tissues were fixed in Zenker's fluid and were routinely stained with phloxine-methylene blue and the Lee-Brown modification of Mallory's aniline blue connective tissue stain. In certain instances elastic tissue and reticulum stains were also employed.

Measurements of the structural components of the alveolus were done with the aid of a micrometer. In order to correct the error due to shrinkage of tissues, the instrument was calibrated in relation to the size of the red cells contained within the lung tissue. The average

diameter of 10 to 20 red cells was accepted as indicating 7.5 microns. The number of capillaries visible in normal and in congested lungs was counted.

Results

I. Structure of Normal Alveolar Wall

Before proceeding to a description of the pathological changes in the minute vessels and corresponding alveolar wall, it would seem

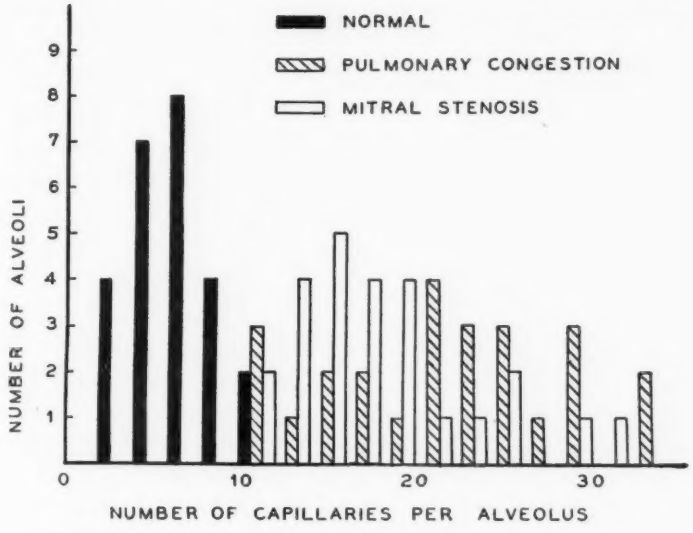

CHART 1

Comparative distribution of the number of visible ("open") capillary lumens per cross-section of a single alveolus in the normal lung, in the lung with simple congestion, and in advanced mitral stenosis

wise to review briefly the normal structure and dimensions of the alveolar wall.

The alveolar wall is covered by a layer of what, in our opinion, are flattened epithelial cells. Beneath these cells is a delicate band of collagen, the so-called alveolar basement membrane. This appears as a homogeneous structure, staining light blue with the Lee-Brown stain. Often placed somewhat eccentrically in the wall and close to the alveolar surface is a capillary whose lumen is sufficiently

wide to admit with ease the passage of a single red cell. In the normal lung the average diameter of the capillary lumen is between 9 and 10 microns. In the absence of congestion one sees relatively few capillaries with a diameter of over 14 microns. Surrounding these capillaries is a layer of collagen, the capillary basement membrane. The thickness of the tissues between the alveolar space and the capillary lumen is between 1 and 2 microns, while that of

TABLE I

Comparison of Diameters of Capillaries and of the Corresponding Alveolar Wall of the Normal Lung and the Lung in Advanced Mitral Stenosis

NORMAL		MITRAL STENOSIS	
Capillary (μ)	Alveolus (μ)	Capillary (μ)	Alveolus (μ)
9	16	20	54
14	17	25	67
9	15	16	45
10	14	10	68
12	16	26	26
9	12	28	55
9	12	20	72
9	14	30	68
10	15	12	70
8	12	7	41

the opposite side of the capillary is between 2 and 3 microns. Ordinarily the alveolar and capillary basement membranes are so close together that they appear as one layer, whereas they are quite separate. This is demonstrated, as will be shown, in instances where there is edema of the wall or an infiltration of cells between the two membranes. In addition to the above components, there are elastic fibers, an occasional delicate bundle of collagen, and a rare fibroblast or histiocyte.

Within the transverse diameter of the alveolar wall there is but 1 capillary. Along the circumference of a cross-sectional surface of the alveolar wall we have counted from 2 to 10 capillary cross-sections, with an average of about 5. Whether these visible cross-sections represent separate capillaries or whether some are parts of the same capillary cannot be stated.

The structure of the normal alveolar wall may be diagrammatically represented, as in Figure 3. Sample measurements of the normal capillary lumen and the alveolar wall, the ratio of the diameter of the capillary to the thickness of the alveolar wall and the relation of these findings to similar measurements obtained in the case of

mitral stenosis are presented in Table I. Chart 1 shows the number of capillaries visible ("open") per cross-section of a single alveolus in a normal lung, as compared with the number visible in congested ones. The difference, which was a consistent finding in counting the capillaries of numerous alveoli, indicates that in the normal lung as compared with the diseased, as observed postmortem, a considerable proportion of the capillaries are collapsed.

II. Structure of Minute Vessels and of Alveolar Wall in Cases with Cardiac and Pulmonary Pathology

Report of a Case

Clinical History: On Dec. 29, 1933, a 33 year old Irish-American female candy worker was admitted to the Boston City Hospital with the history of "heart trouble" of 2½ years duration.

At the age of 10 years the patient was out of school for 6 months with a "nervous condition," probably chorea. At the age of 15 she suffered from generalized joint pain, with tenderness, swelling and redness. This condition lasted but 1 or 2 weeks. Six years before admission to the hospital she developed dyspnea on exertion, and suffered from coughing spells 5 to 6 times a year. About 2½ years ago a heavy tray was dropped on the patient's head. Within a few hours she developed severe cyanosis, precordial pain and orthopnea. The pain radiated to the left side of the back and was accompanied by a sensation of heat down the left arm. Ever since this episode the patient had been an invalid, confined to bed during the greater part of the day. On several occasions she had been troubled by attacks of severe dyspnea, orthopnea, palpitation and precordial pain. During the past year, on 5 occasions she experienced severe attacks of a choking sensation with obligatory orthopnea, followed by rather profuse hemoptysis. On each occasion the sudden hemorrhage was followed by the raising of dark clots and streaked sputum for 3 or 4 days. During the week before she entered the hospital the patient became intensely dyspneic and felt chilly.

The family, social and past histories contained no additional pertinent facts. There was no history of nocturia or of edema. The patient had lost some 12 kg. during her illness, weighing 42 kg. at entrance to the hospital. Her height was 157 cm.

Physical Examination: On admission she appeared rather poorly nourished, and had to be propped up in bed. She was quite dyspneic, the lips were intensely cyanotic and her condition seemed alarming. The head and neck were normal without evidence of venous congestion. The chest showed symmetrical and somewhat limited excursion. The lungs were resonant with the exception of the right base, which was somewhat dull. Anteriorly over the subclavicular area, moist râles, and over the axillae and posteriorly toward the base, crackling râles were heard.

The apex impulse of the heart was in the fifth space, where the maximal left border was 11 cm. A systolic thrill was felt in the fourth space on the left side of the sternum. The first cardiac sound was marked by a presystolic murmur

over the apex. There was also a diastolic murmur and a snapping second sound. A gallop type of rhythm with a third heart sound and with a markedly accentuated second sound was heard. The pulmonary second sound was also accentuated and coincidentally a "shock" was felt over the pulmonary conus. The pulses were small. The arterial pressure was 60/30 mm. Hg. on admission; later the average level was 110/60 mm.

The liver edge was felt two fingers below the costal margin. There was no subcutaneous edema. The results of the rest of the examination were irrelevant.

Course of Illness: During almost 3 months stay in the hospital the patient suffered from numerous attacks characterized by intense dyspnea, orthopnea and accentuated cyanosis. On such occasions the lungs filled up with bubbling moist râles and she expectorated frothy, blood-tinged fluid. The patient became livid blue and later ashy gray. On each occasion she went into circulatory collapse. The attacks were relieved with oxygen and caffein. Between attacks she remained propped up in bed. During the first half of her last illness in the hospital the lungs cleared up fairly well between the attacks of dyspnea and pulmonary edema, but during the last 6 weeks of life coarse bubbling râles were present throughout the lung fields, particularly through the *upper part of the lungs*. Signs of fluid appeared in the right pleural cavity about 6 weeks before death, and on four occasions thereafter 1300 to 1500 cc. of amber colored fluid were removed, with temporary relief. Slight edema of the ankles appeared during the last 2 weeks. The patient expired on March 17, 1934, following an attack of dyspnea.

The temperature was essentially normal during the first 4 weeks; thereafter it rose to 101° F. at irregular intervals. The heart rate fluctuated between 80 and 120 per minute, and the respirations from 20 to 30.

Laboratory Data: X-ray examination of the chest (Dec. 29, 1933) revealed rheumatic deformity of the heart with congestive changes in both lungs. On Jan. 18, 1934, the diameter of the great vessels was 5 cm., the maximal transverse diameter of the heart 13.8 cm., and of the thorax 24 cm. Evidence of a small amount of fluid was present over the right base. On Jan. 24, 1934, in addition to the previous findings, localized shadows over the left axilla suggested small infarctions or pneumonia.

On Dec. 29, 1933, and on Jan. 25, 1934, the electrocardiogram revealed normal sinus rhythm. The P-R interval was 0.16 second, the Q-R-S 0.08 second. T_1 was flat, T_2 and T_3 inverted. The axis indicated right ventricular preponderance. On March 6, 1934, the electrocardiogram revealed auricular fibrillation.

Numerous analyses of the urine failed to reveal abnormal findings. The result of the concentration and dilution test of the urine was normal. The non-protein nitrogen of the blood was 41, 37 and 35 mg. per 100 cc. on different occasions. The Kahn test of the blood was negative. The red blood cell count varied between 4,000,000 and 5,200,000 per cubic millimeter, and the hemoglobin ranged from 69 to 84 per cent. The hematocrit reading was 42 per cent, mean corpuscular hemoglobin concentration 31.4 per cent, mean corpuscular hemoglobin 25.3 micrograms, mean corpuscular volume 80 cubic microns. The platelets were essentially normal and there was slight achromia. There was a continuous slight leukocytosis, 10,800 to 15,700 white blood cells per cubic millimeter, which on two occasions rose to 28,700 and 22,000. The polymorphonuclears varied from 63 to 86, lymphocytes 10 to 24, monocytes 4 to 10, eosinophils 1, basophils 2 per cent. Blood cultures were sterile. The chest fluid showed a specific gravity of from 1.006 to 1.012, red blood cells 2500 to 9000, white blood

cells 160 to 2200 per cubic millimeter; polymorphonuclear leukocytes predominated. The non-protein nitrogen content of the pleural fluid was 20 to 29 mg. per 100 cc.; protein 1.1 to 1.5 gm. per 100 cc.

Clinical Diagnoses: Rheumatic heart disease with advanced mitral stenosis; paroxysmal dyspnea (cardiac asthma) with attacks of acute pulmonary edema; chronic passive congestion and chronic edema of the lungs; right hydrothorax and congestive failure of the circulation; pulmonary infarcts.

Autopsy Report

Gross Findings: The examination was performed 5 hours postmortem. There was intense cyanosis of the lips and face, and marked lividity of the dependent parts. Slight pitting edema was present up to the knees. The *peritoneal cavity* contained 1000 cc. of clear amber fluid. The liver edge was 17 cm. below the xiphoid base, and 8 cm. below the costal margin on the right. The right *pleural cavity* contained 800 cc. of clear fluid; the left was obliterated by firm fibrous adhesions. The *pericardial cavity* contained 200 cc. of fluid.

The *heart* weighed 380 gm. The trabeculae of the right ventricle were moderately thickened. The endocardium of the left auricle was white and opaque and the auricular myocardium presented trabeculation. The mitral valve was rigid and sclerotic. The opening was represented by a narrow slit, 2 cm. long and 2 mm. wide (Fig. 1). The edges of the leaflets were slightly overlapping, and there was evidence of fusion of the leaflets at both edges of the fissure. The chordae tendineae were greatly thickened, shortened and calcified. The trabeculae of the left ventricle were markedly thinned. The aortic cusps showed questionable slight fusion at the commissures. The other valves were normal. The coronary arteries were also normal.

The cardiac measurements were as follows: tricuspid circumference 11 cm.; pulmonary opening 6.5 cm.; mitral slit 2 cm. long and 0.2 cm. wide; aortic opening 6 cm. The thickness of the left ventricle was 0.8 to 1.4 cm., and that of the right 0.2 to 0.5 cm. The aorta was delicate and elastic. There were traces of yellow atheromatous streaking in the lumbar region. The circumference measured 6 cm. at the ring, 3.5 cm. at the upper dorsal level, 3.4 cm. at the first lumbar vertebra, and 2.5 cm. at the bifurcation.

The *lungs* weighed 1350 gm.; the right 700 gm. and the left 650 gm. The pleural surface on the right was glistening, and on the left

contained fibrous tags. The cut section presented a rather unusual picture. The lower halves of both lungs were gray and somewhat firm and dense, though crepitant; they contained little blood. The upper halves were red and intensely engorged (Fig. 2). Over the upper half and middle portion of the right lung there were several small infarcts, one of which was located at the tip of the apex. The trachea and bronchi were dull brownish red. The pulmonary artery was somewhat dilated and was of normal elasticity, but the intima revealed numerous, rather soft, yellow atheromatous patches.

The *liver* weighed 1460 gm. The surface was slightly granular. The vascular markings were intensified.

The rest of the postmortem examination, except for evidence of passive congestion, was irrelevant.

Anatomical Diagnoses: Rheumatic heart disease with an unusually high degree of stenosis and calcification of the mitral valve; pulmonary congestion and edema of the upper half, and induration of the lower half of the lungs; small pulmonary infarcts; right hydrothorax; obliterative healed left pleuritis; ascites; passive congestion of the liver; edema of the gall bladder; chronic cystitis and vaginitis.

Microscopic Description

In describing the pathological changes in the *lungs*, their various component parts will be taken up separately:

1. *Alveolar Walls:* The severity of the lesions varied with the different lobes and portions of the lobes. In general, the lower lobes showed the most marked changes, particularly the lower parts of these lobes. Also, the lower portions of all the lobes were involved more markedly than the upper.

The least severe change noted was a marked dilatation of the capillaries, the diameters of their lumens being five or six times that of a red cell. In regions where the lesions were more advanced, not only were the capillaries dilated but they also appeared increased in number. This apparent increase may well have been due in part to increased length or to tortuosity. Often such capillaries presented an aneurysmal dilatation and bulged out into the alveolar space (Fig. 5). Accompanying this stage, the capillary basement membrane frequently showed slight thickening, but the alveolar basement membrane was normal.

A greater degree of change consisted in an increase in thickness of the capillary basement membrane and also of the interstitial collagen; the alveolar basement membrance even at this stage was normal. The capillaries here were dilated but showed less herniation into the alveolar spaces (Fig. 6).

Where the process had advanced further there was a definite increase in the interstitial connective tissue, and the capillary basement membrane was markedly thickened while the alveolar basement membrane remained normal. At this stage the capillaries were usually separated by the interstitial collagen from the alveolar space, which tended to be lined with cuboidal cells (Fig. 7). These cells often contained fat vacuoles. Thus the capillaries were separated from the alveolar space by structures of considerable thickness. Finally, the capillaries in such thickened walls became small, and in some none could be made out. Even in these advanced stages the alveolar basement membrane was never thickened, in contrast to the capillary basement membrane. These advanced structural changes were common findings in the lower third of the lungs.

Edema of the interstitial tissue of the wall was not uncommon. This was evidenced by separation of the component parts by fluid, with resulting thickening of the wall. The alveolar basement membrane was ballooned outward and it was in this condition that this membrane could best be recognized (Figs. 8 and 9). Such interstitial edema was by no means always accompanied by edema of the alveolar spaces.

The elastic tissue showed no definite changes.

There was often an infiltration of lymphocytes and macrophages in the thickened walls, and diapedesis of red blood cells into the walls was frequently seen. The alveolar spaces contained varying numbers of pigmented macrophages, and also edema fluid. In some areas emphysema was present. This led to narrowing of the alveolar wall with resultant diminution in the capillary lumens, often of such a high degree that a single red cell could barely pass through. In some such walls there was thickening of the collagen, indicating that congestion had existed here before the emphysema developed (Fig. 10).

A section through an infarct showed a picture, the significance of which will be discussed later. The parenchyma adjacent to the

infarct exhibited the most marked changes described above; *i.e.* great thickening of the alveolar walls with the air spaces lined by cuboidal epithelium. The alveolar walls in the infarcted area, on the other hand, showed slight if any increase in collagen and were

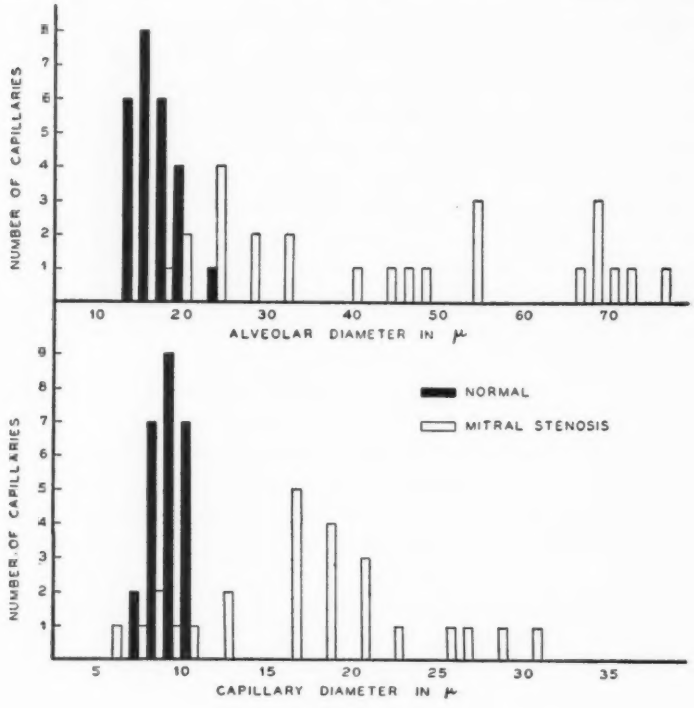

CHART 2

Sample of comparative measurements of the diameters of 25 alveolar walls and of corresponding capillary lumens of the normal lung and of the lung in advanced mitral stenosis

essentially normal as far as the connective tissue content of their walls was concerned. The condition of the smaller arteries and arterioles in the infarcted area could not be determined, since all cellular elements were, of course, completely necrotic.

The diameters of several hundreds of capillary lumens and of alveolar walls have been measured. Table I shows examples of such

measurements. There was usually a striking increase in both of these measurements, but the changes within the same alveolus did not parallel each other. Over the basal portion of the lungs, capillaries of normal diameter (7 to 9 microns) were often embedded centrally or eccentrically in alveolar walls of 40 to 60 microns in thickness, containing collagen staining intensely blue with the aniline blue stain. Chart 2 presents comparative measurements of the diameters of the alveolar walls and of the corresponding capillary lumens.

An attempt was made also to count the number of capillary lumens visible along the cross-section of an average sized alveolus. Chart 1 presents sample data of such counts in a normal lung, in a case of simple pulmonary basal congestion without heart disease, and in the case with mitral stenosis. As indicated, three to four times as many capillaries were visible in the presence of pulmonary congestion. It is of interest that the increase in the simple pulmonary congestion was of the same order of magnitude as in the case with mitral stenosis with intense active and passive congestion. Obviously, the number of capillary lumens visible along the cross-section of the alveolus does not correspond with the number of capillaries within the alveolus. If a capillary is tortuous the section may contain several cross-sections of the same capillary. Such counts as are presented in Chart 1 indicate, nevertheless, that in the normal lung (postmortem) the majority of the capillaries are not filled with blood unless there is active or passive congestion. Judging from the histological appearance of the capillaries and from the capillary counts, in the case of mitral stenosis not only were all the available pulmonary capillaries filled with blood, but they were stretched to their maximal capacity.

2. *Arteries:* (*A*) *Large Arteries:* These vessels showed marked thickening of the intima as a result of proliferation of connective tissue, which in its deeper portions often contained large, lipoid-filled macrophages. Reduplication and splitting of the internal elastic laminas were present. Thrombi undergoing various stages of organization were common.

(*B*) *Medium Sized Arteries:* These presented an unusual picture. The early lesion apparently consisted of a subendothelial deposition of fibrin with an associated proliferation of the lining endothelial cells (Fig. 11). Accompanying this stage was an increase in the

intimal connective tissue, in which appeared a number of capillaries. In the late stage the lumen was represented by narrow endothelial-lined spaces and the intima by vascular connective tissue (Fig. 12).

(*C*) *Small Arteries:* Marked intimal thickening due to increase in connective tissue and also some medial hypertrophy characterized the changes in this group. The alterations of the intima were the more striking, and resulted in a disproportion between the thickness of this layer as compared with that of the media.

(*D*) *Arterioles:* The walls of these vessels showed a high degree of thickening with apparent diminution in the diameters of their lumens. This thickening was due to a concentric proliferation of the cells making up their walls (Figs. 13 and 14). Between these cells lay delicate bands of collagen. In addition, a rare vessel undergoing necrotizing arteriolitis could be found (Fig. 15). Such arterioles showed blurring of the outlines of their walls with karyorrhexis of the nuclei and diapedesis of red cells. This type of lesion was also noted at the periphery of an infarct of some age. Hyalinization of the arterioles was not seen.

3. Veins: The only change of note was a thickening and hyalinization of the surrounding collagen.

4. Interstitial Connective Tissue: Edema of the septa was present in a marked degree without an associated edema of the alveolar spaces (Fig. 16). There were several foci of myelocytes and nucleated red cells in the perivascular and pleural connective tissue. The pleura showed marked increase in connective tissue.

Heart: There was some fibrous thickening of the epicardium with an infiltration of lymphocytes. In the myocardial connective tissue a rare Aschoff body and a few lymphocytes were noted. There was a minimal amount of scarring. Branches of the coronary arteries were thickened.

Liver: Hemorrhagic central necrosis, both old and recent, was present.

The other organs revealed nothing remarkable on postmortem examination.

Summary

The main features of this case may be summarized as follows:

1. An unusually advanced degree of mitral stenosis was associated with attacks of dyspnea, orthopnea and pulmonary edema.

During the last 4 weeks of life the patient exhibited a constant state of pulmonary edema and congestion. Clinical evidence of failure of the peripheral circulation appeared only as a terminal manifestation.

2. Because of the intense basal pulmonary congestion and edema and probable secondary infection, a severe degree of induration of the lower half of the lungs developed. This induration consisted mainly in a marked degree of thickening of the alveolar walls due to increased connective tissue and to a change of the flat epithelial layer into a cuboidal one. As a result of these changes the pulmonary capillaries became compressed; many of them were displaced into the middle portion of the alveolar wall and became surrounded by thick layers of collagen. Several of the alveoli contained no capillaries.

3. As a result of the above changes over the lower half of the lungs the congestion over the upper part of the lungs became intensified. Here, including even the apical area, the capillaries became widely dilated, many of them showing aneurysmal dilatation and bulging into the alveolar spaces. The capillary basement membrane showed various degrees of thickening, in contrast to the unchanged alveolar basement membrane. Separation of the two basement membranes by pericapillary edema was frequently observed.

4. There was atherosclerosis of the larger pulmonary arteries, healing and healed rheumatic arteritis of the medium sized arteries, and hyperplastic arteriolosclerosis and rarely necrotizing arteriolitis of the arterioles. It was particularly significant that both the frequency and the degree of the arteriolar changes increased from the apex towards the base.

5. The main changes in the veins consisted in increased connective tissue surrounding them.

6. Edema of the interlobular septa occurred independently of alveolar transudation.

Findings in Other Cases

In order to ascertain the significance of the pulmonary and vascular lesions observed in the case described, we have studied in a similar detailed manner 9 additional cases with mitral stenosis of rheumatic origin. Four cases in this group showed alveolar and vascular changes similar to those found in the first case but without rheu-

matic arteritis. In the remaining 5 cases the lesions were much slighter, consisting mainly of some thickening of the alveolar walls with a moderate degree of increase in the collagen and with distended capillaries over the lower portion of the lungs; there were no changes in the small arteries and the arterioles.

A comparison of the clinical and gross morphological characteristics of the 5 cases with advanced pulmonary vascular and parenchymatous lesions with those of the 5 cases with mainly intense pulmonary passive congestion revealed the following features: Four of the 5 subjects with marked pulmonary and vascular changes were young, the ages varying between 26 and 36 years. The age of the 5th subject was 58 years. The first rheumatic infection and the discovery of heart disease in each case dated back to childhood, but in all cases manifestations of a pronounced degree of cardiac failure appeared within 1 to 3 years of death. The circulatory failure was characterized mainly by intense cyanosis, complete physical disability associated with progressive and continuous severe dyspnea and orthopnea, precordial oppression and pain, with or without radiation, and later superimposed attacks of paroxysmal dyspnea and hydrothorax. The patients complained of periodic attacks of severe cough, "bronchitis" and hemoptysis. All these symptoms were present at first without evidence of peripheral congestive failure, such as venous engorgement, ascites, enlargement of the liver, jaundice, and edema of the lower extremities. Such manifestations developed relatively late. Once advancing cardiac failure appeared, the patients responded poorly to treatment. The cardiac rhythm was regular or auricular fibrillation. The first cardiac sound was loud and the pulmonary second sound accentuated and reduplicated. Other cardiac manifestations of mitral stenosis were also present. The electrocardiograms in three instances revealed high P waves. The contour of the X-ray picture revealed mitral bulging and varying degrees of increased pulmonary conus. The clinical features of these patients corresponded in several aspects to those described by Held, Goldbloom and Lieberson,[5] but the degree of cyanosis and respiratory difficulties were more intense.

Postmortem examination revealed a slit shaped, narrow mitral orifice in 4 cases. In the 5th case there was stenosis admitting the tip of the little finger. In all 5 cases the narrowed and distorted mitral leaflets were sclerosed and calcified and the chordae tendineae

were shortened and thickened. Acute rheumatic verrucous endocarditis was not present. The other valves were normal except in 2 cases in which the edges of the aortic cusps were "rolled" and slightly thickened. The weight of the heart was slightly or moderately increased. The left ventricle was of normal size and thickness, but the other chambers of the heart showed pronounced dilatation as well as thickening. In each of the 5 cases mural auricular thrombi were present. One or both of the pleural cavities contained from 1 to 2 liters of fluid. The peritoneal cavity was either filled with fluid or normal. The large pulmonary arteries showed a slight degree of sclerosis. The bases of both lungs in each of the 5 cases were gray, the consistence was increased and firm, and they contained a decreased amount of air. The upper portions of the lungs were red and congested and contained one or more small red infarcts. The severity and extensiveness of the basal induration and congestion of the upper portions varied, but in none of the 4 cases was the degree as severe as in Case 1.

The ages in the other group of 5 cases were more advanced. The onset of the rheumatic infection dated back to youth, and the duration of heart disease was longer. The onset and the progress of the symptoms of advancing cardiac failure were gradual, and there was a lesser degree of dyspnea, orthopnea and suffering in general than in the group previously described. These patients did not suffer from paroxysmal dyspnea. Cyanosis was either slight or absent. The clinical manifestations of pulmonary and peripheral congestion appeared simultaneously.

Postmortem examination showed a healed, scarred and calcified mitral valve admitting the tip of one finger. The chordae tendineae were shortened and thickened. In addition to the mitral stenosis, 1 case showed slight aortic stenosis, 1 a moderate degree of tricuspid stenosis and 1 aortic insufficiency. The degree of cardiac hypertrophy was greater and the auricular dilatation less than in the previous group.

The pleural cavities contained no fluid or only moderate amounts; the peritoneal cavity was either partially or completely filled with fluid. The large pulmonary arteries showed a moderate degree of sclerosis. The lungs exhibited basal congestion.

In addition to the detailed study of these 10 cases, the routine histological sections of 13 cases with mitral stenosis of varying de-

gree associated with other types of rheumatic cardiac involvement were examined, but sections of only 2 of these cases exhibited diffuse sclerosis of the small arteries and of the arterioles. A certain degree of thickening of the alveolar wall was frequently observed.

Control Cases

It is of interest that sections of the lungs from 5 cases of congenital cardiac septal defect, 12 cases of congestive circulatory failure of luetic and hypertensive origin, 15 cases showing pulmonary emphysema, 1 case of marked kyphosis and sclerosis of the larger pulmonary arteries, 20 cases of thrombosis or embolism of the pulmonary arteries, 19 cases showing chronic interstitial pneumonitis, and 3 cases of pulmonary fibrosis of non-cardiac origin failed to reveal pulmonary vascular and parenchymatous changes similar to those in the 5 cases that showed advanced mitral stenosis.

Through the courtesy of Dr. Tracy B. Mallory we have also examined the slides from a case diagnosed as Ayerza's disease (No. 6248). In this case, however, the small vessels were not involved and the alveolar structure was normal. Intimal proliferation and occlusion of several of the larger vessels were the main findings.

Discussion of Pathological Findings

The changes in the alveolar walls described above are in essential agreement with those reported by zu Jeddeloh.[3] Such changes are presumably the result of long continued hypertension, stagnation and edema. As a result of the increased intravascular pressure there occurs first a dilatation and apparent elongation of the capillaries. Following this there is a thickening of the capillary basement membrane, and this finally is followed by an increase in the interstitial collagen of the alveolar wall. This latter change we consider the result of interstitial edema.

As a result of the increased connective tissue and the consequent separation of the capillaries from the alveolar spaces, the involved alveoli are no longer capable of functioning. This is indicated by the cuboidal type of cells lining the air sacs. Function is also interfered with by the interstitial edema which fills and increases the space between the capillaries and air sacs. Furthermore, at various areas emphysema results in a marked diminution in the caliber of the cap-

illary bed, due to stretching with resulting narrowing of the alveolar walls. The diameter of the capillaries in such thin alveolar walls is frequently less than normal, and this narrowed capillary bed provides an increased resistance to the blood flow. It is of interest to note that emphysema occurred in some regions where apparently chronic congestion had existed previously, as indicated by the increased amount of collagen in the alveolar walls.

The vascular changes varied with the size of the blood vessels in question. The larger arteries showed proliferative changes in the intima with, in addition, the occurrence of lipoid-filled macrophages. The intimal proliferative changes consisted of increased connective tissue with splitting and reduplication of the internal elastic laminas. Such changes have for many years been ascribed to the effect of hypertension, and in our opinion are rightly so interpreted, since analogous lesions are seen in the kidney in cases of hypertension.

The changes in the medium sized arteries described in our first case are to be regarded as probably of rheumatic origin, in view of the studies of VonGlahn and Pappenheimer.[6] We observed such lesions in none of our other cases. In them, the medium sized and smaller arteries showed marked fibrous thickening of the intima of such a degree that often the intima was thicker than the media. There was also some hypertrophy of the media.

The arterioles presented the picture of hyperplastic arteriolosclerosis (productive endarteritis), *i.e.* thickening of the walls due to concentric cellular proliferation, giving an onion-like layer of cells in the walls. This vascular change especially aroused our interest because of its similarity to the arteriolar changes in the kidney in malignant hypertension. This similarity was further emphasized by the occurrence — rare, it is true — of necrotizing arteriolitis. This lesion, in addition to being present in scattered areas in the lung, was found also in the vicinity of an infarct, a fact the significance of which for renal infarcts has been discussed by Klemperer and Otani[7] and more recently by Kimmelstiel and Wilson.[8] No hyaline degeneration of the arterioles such as is seen in the kidney, pancreas and adrenals in benign hypertension was noted in the lungs in our series.

In reviewing the vascular changes in the lung described above, one is struck by their similarity to the changes observed in the kidney in malignant hypertension. In both organs the larger arteries

show proliferative changes in the intima. The arterioles show hyperplastic arteriolosclerosis and in addition necrotizing arteriolitis In view of this, it seemed of interest to determine approximately the dimensions of the vessels showing these various changes in the two organs under discussion. For this purpose, the external diameters of the vessels were measured. In both organs it was found that the great majority of vessels showing hyperplastic arteriolosclerosis and arteriolonecrosis measured less than 100 microns, while those showing intimal proliferative changes measured more than 150 microns.

The vascular changes in both organs lead to serious interference with their functional units, *i.e.* the glomerulus and the alveolar wall. These two structures have in common certain anatomical and physiological functions. Both are made up of capillaries, surrounded by a basement membrane which is covered by epithelium. As a result of vascular alterations, both units undergo changes which eventually result in the complete loss of function. In the kidney the glomerulus becomes a hyalined scar; in the lung the alveolar wall a broad band of rather avascular connective tissue.

Finally, we feel that the histological picture found in the infarct in Case 1 is important from the point of view of the length of time necessary to produce the pulmonary changes we have described. As pointed out in the microscopic description, the alveolar walls in the infarct showed no increase in connective tissue, while the adjacent walls exhibited a marked degree of thickening and the alveolar epithelium was cuboidal in type. There are apparently two explanations of the above picture: (1) the infarct involved normal tissue, and (2) the infarct occurred before the parenchymatous changes had taken place. Against the first suggestion is the fact that all the surrounding parenchyma is pathologically altered and it seems improbable that there should be a directly contiguous area of normal tissue, since the process in any one given area tends to be uniform and diffuse. If the second explanation, which seems the more probable, is correct, then we have definite evidence that the changes in the alveolar walls can occur in a comparatively short period of time, for the infarct shows but little signs of healing. In our estimation the duration was weeks rather than months.

Clinical and Physiological Significance of Structural Alterations in the Lungs

The clinical manifestations of failure of the pulmonary circulation are believed to depend on a dynamic alteration of the blood flow secondary to the back pressure effect of cardiac lesions or dysfunction in the left side of the heart. The physiological changes manifest themselves primarily in retardation and stagnation of the flow, which is more marked over the lower than over the upper portions of the lungs.[2, 9, 10, 11] The observations here presented, however, demonstrate that the circulatory engorgement can induce permanent structural alteration of various tissue components of the lung, which, in turn, can then be the source of disturbed functions, as well as of clinical symptoms and signs. *The nature of these structural changes is such that they interfere with the vital pulmonary function of gaseous exchange.* Over the lower half of the lungs the capillary basement membrane becomes thickened and deposition of a considerable amount of collagen separates the capillary lumen from the alveolar space. Not infrequently the flat, thin epithelial cells become thickened and cuboidal. The normal thickness of 1 to 3 μ of the alveolar tissue, through which oxygen and carbon dioxide have to diffuse, can change to a thickness of 30–50 μ. Over such pulmonary areas the blood flow is shunted without any appreciable degree of alteration of its gaseous contents. In advanced cases of "tight mitral stenosis," thickening of the alveolar wall with markedly dilated capillaries may be present not only in the lower but also, as has been shown, in the upper portion up to the apex. In spite of the fact that even in these high non-dependent areas the pericapillary collagen can be somewhat increased, both oxygen and carbon dioxide obviously must diffuse freely through these alveolar structures. Nevertheless, the fact that the diameters of the *distended capillaries* permit the simultaneous passage of from five to twenty or more red cells, instead of one or two, must be a significant contributory factor to the maintenance of arterial anoxemia. It is, however, of interest that study of the arterial blood in patients with circulatory failure often indicates no increase in the carbon dioxide content, even in the presence of a severe degree of anoxemia.[12] This difference in oxygen and carbon dioxide content must be explained by the greater diffusion coefficient of carbon dioxide. Liljestrand and

Sahlstedt[13] have found that carbon dioxide diffuses about forty times more rapidly than oxygen through the alveolar wall of the lung of the frog.

Patients with chronic heart disease, particularly with mitral stenosis, at times exhibit intense cyanosis regardless of subsequent myocardial improvement, as indicated by studies of hemodynamics. The structural alterations in the pulmonary architecture observed in this study suggest that if, as a result of long existing pulmonary engorgement, the structural alterations are permitted to develop, certain symptoms such as cyanosis and even dyspnea may persist regardless of the myocardial improvement established thereafter. The change in size and in the elastic properties of the lung tissue in these cases must represent, for the development of dyspnea and tachypnea, a stimulus to the nerve endings located in the lungs and in the pleura similar to that present in chronic emphysema or interstitial pneumonitis. The finding of thickened alveoli exhibiting increased amounts of collagen and other changes demonstrates that in the presence of chronic failure of the circulation not only physiological but also structural alterations contribute to the *stiffening of the lungs* ("Lungenstarre" of von Basch[14]).

Some of the patients whose lungs were studied exhibited rather profuse transient pulmonary hemorrhages. The rupture of the large bulging capillaries observed is an obvious source of such hemorrhages. Whether diseased arterioles rupture at times, we do not know.

We have been impressed in the past by the frequent lack of correlation between acute or chronic dyspnea and orthopnea, on the one hand, and such clinical signs as pulmonary râles, on the other hand. On postmortem examination one observes heavy lungs with only a moderate degree of hyperemia and no appreciable amount of edema within the air passages. The demonstration of the existence of *pericapillary edema* without intra-alveolar edema offers adequate explanation for such a situation, and the findings presented demonstrate also that a considerable amount of tissue fluid may accumulate around the capillary bed. Such a state of affairs must contribute to the patient's distress, and this type of edema, in contrast to other structural changes, should be amenable to therapeutic procedures. We have also observed not uncommonly the opposite situation, namely, the existence of *intra-alveolar edema* without capillary en-

gorgement and without alteration in the structure of the alveoli. In this type of pulmonary edema a primary alteration of the permeability must be the determining factor. Finally, pericapillary and intra-alveolar edema often coexist.

When both primary pulmonary *emphysema and heart disease* were present, the capillary bed usually was not dilated. In these cases, although the alveolar wall shows no thickening or only a slight degree of thickening, an increase in the collagen content of the alveolar wall has been demonstrated. Hence, here, too, the circulatory engorgement contributes through structural alterations to the further rigidity of the alveoli. The fact that in an emphysematous lung the already diminished capillary bed cannot dilate, represents loss of an important vascular reserve function of the lung, which is essential under certain types of stress. Loss of such reserve function must facilitate the development of pulmonary hypertension.

In attempting to throw light on the etiology of pulmonary arteriolosclerosis, it seemed significant that the advanced vascular lesions were situated in the lower portions of the lungs, while in the upper portions sclerosis was not present or was but slight. This striking difference in the distribution of the vascular lesions must bear pertinently on the origin of the type of sclerosis described. The main differential characteristics of the functional state of the lower portions of the lungs as contrasted with those of the upper portions in the cases studied were: (1) higher capillary and arteriolar pressure; (2) slower blood flow and stagnation; and (3) pericapillary and intra-alveolar edema. It is therefore rational to conclude that the vascular changes observed are dependent on these three factors, or on chemical or morphological alterations which are secondary to these factors. It is of significance, also, that in malignant nephrosclerosis, high pressure, as well as stagnation, is present within the arterioles exhibiting sclerosis, as is indicated by injection methods.[15]

That combination of these three factors seems to be essential is shown by the fact that in pathological conditions where only one of the factors is present, arteriolar sclerosis, and particularly necrotizing arteriolitis are not observed. Thus the clinical and histological examinations of the upper portion of the lung in Case 1 have indicated markedly increased vascular pressure, but vascular sclerosis was not present. In the control group of pulmonary emphysema and congenital heart disease with hypertrophied right ventricle, sclerosis

was also absent, in spite of the fact that the pulmonary blood pressure was presumably high. Similarly, in a group of cases with long persisting chronic passive congestion and edema of cardiac origin, or with chronic pneumonitis and fibrosis, the vascular lesions were not present. On the other hand, it has been shown recently that long persisting pulmonary edema induced in the rat by the prolonged administration of oxygen under high barometric pressure can induce a type of pulmonary arteriolosclerosis consisting of a thickening and hyalinization of the walls with ultimate thrombosis of many.[16] It is of interest that in addition to engorgement and edema, the lungs of these animals also exhibited increased arterial pressure.[17] These studies are of particular interest because they rule out anoxemia as an etiological factor.

It would be of significance to be able to estimate the time element essential for the development of the arteriolar lesions described. It is therefore pertinent that when the patient in Case 1 entered the hospital the lung fields were essentially of normal density, as indicated by X-ray examinations, and the marked degree of density of the lower half of the lungs developed during a period of $2\frac{1}{2}$ months. It was also of significance that the red infarcted areas contained no thickened alveoli, while the non-infarcted areas at a corresponding level exhibited all grades of parenchymatous changes. As these infarctions were not older than 1 or 2 months, the advanced pulmonary lesions must have developed within the same period of time. That in malignant nephrosclerosis similar types of lesions can develop in the kidney within a period of the same order has been actually demonstrated by us in a comparative study of the two kidneys of the same case obtained at an interval of 67 days.[18] Finally, it has been shown that pulmonary arteriolosclerosis and an increase in the interstitial collagen can be induced experimentally within 30 to 40 days.[16]

Advanced ("tight," "non-regurgitant," "buttonhole") mitral stenosis is but one type of lesion that can lead at times to the combined presence of the three pulmonary factors mentioned. In cases with chronic pulmonary emphysema or congenitally increased pulmonary vascular resistance in which repeated attacks of pneumonitis or other types of change associated with edema develop, the three responsible factors are particularly apt to coexist and hence pulmonary arterial and arteriolar sclerosis are expected to occur.

Such may indeed be the etiological mechanism in instances described as primary pulmonary sclerosis of "Ayerza syndrome."

The 5 cases of hyperplastic and necrotizing arteriolitis exhibited intense dyspnea, orthopnea, tachypnea, cyanosis and precordial oppression and pain. Superimposed on these difficulties were attacks of severe cough, hemoptysis and cardiac asthma with transient intra-alveolar edema. The pulmonary second sound was loud and reduplicated. These manifestations are indicative of unusually high pulmonary pressure. The postmortem examination likewise suggested high pressure throughout the pulmonary circuit.

It has also been shown in this study that these cases of advanced mitral stenosis exhibited vascular changes quite similar to those found in the arterioles of the larger circuit, particularly in the kidneys of patients with malignant arterial hypertension. It has also been stressed earlier that a number of similarities exist between the function and structure of the glomerulus and alveolus. While in the presence of advanced mitral stenosis the pulmonary vascular system is involved and the arteriolar system of the larger circuit is normal, in cases of uncomplicated malignant hypertension of the larger circuit the situation is reversed, and the vascular system of the pulmonary circuit is normal. These clinical and physiological considerations, together with the morphological findings here presented, indicate a close similarity existing in the pulmonary circuit in the group of cases of mitral stenosis, and in the larger circuit in the group designated as malignant hypertension with malignant nephrosclerosis.[18] Hence these advanced cases of mitral stenosis can be considered as exhibiting the clinicopathological syndrome of *pulmonary hypertension with malignant sclerosis*. This designation does not imply that the syndrome is always clear-cut, or that clinically it can always be sharply differentiated from the syndrome of pulmonary hypertension without advanced or malignant sclerosis. In this respect, too, the problem of the malignant sclerosis of the two vascular circuits is identical.

Because the mechanical and circulatory factors active in this type of mitral stenosis leading to malignant pulmonary sclerosis are known to a large extent, further studies on the origin of these pulmonary vascular lesions may well throw light on the etiology of vascular changes in malignant hypertension and malignant nephrosclerosis.

Summary and Conclusions

1. The changes in the blood vessels and alveolar walls in the lungs in cases showing an advanced degree of rigid mitral stenosis associated with intense failure of the pulmonary circulation are presented, and the structural alterations found are compared with those observed in other types of mitral stenosis and in a control group with varied types of cardiac and pulmonary disease.

2. The lesions in the pulmonary vessels consisted of (*a*) intimal thickening of the arteries, and (*b*) hyperplastic arteriolar sclerosis and arteriolar necrosis.

3. The changes in the alveolar walls consisted of (*a*) marked dilatation of the capillaries, (*b*) increase in the thickness of the capillary basement membrane, (*c*) increase in the interstitial tissue (collagen), (*d*) interstitial pericapillary edema, and (*e*) a tendency of the flat epithelial cells to become cuboidal in shape.

4. The normal thickness of 1 to 3 μ of alveolar tissue through which oxygen and carbon dioxide have to diffuse can increase up to a thickness of 30–50 μ.

5. Even in the presence of an advanced degree of thickening of the alveolar wall and of the capillary basement membrane, the alveolar basement membrane remains normal.

6. With progressive pulmonary engorgement, first the visible capillaries increase in number, and only later do they dilate. Often the capillaries become displaced and are separated from the alveolar surface by a considerable degree of edema or by thick layers of collagen.

7. Pericapillary and intra-alveolar edema frequently develop independently.

8. Permanent structural alterations in the lungs caused by circulatory failure interfere with the gaseous exchange, partly through altered permeability of the alveolar wall, and partly as a result of the simultaneous passage through the individual capillaries of numerous columns of red cells, instead of a single red cell.

9. In the causation of the pulmonary arterial and arteriolar lesions, an important rôle is played by the prolonged combined presence of (*a*) high intravascular pressure, (*b*) stagnation of blood flow, and (*c*) edema.

10. Evidence is presented that advanced vascular lesions in the

pulmonary, as well as in the larger circulation, can develop within about 2 months.

11. The clinicopathological syndrome of pulmonary hypertension with "malignant" sclerosis is described and the similarity between this condition and arterial hypertension with malignant nephrosclerosis is discussed.

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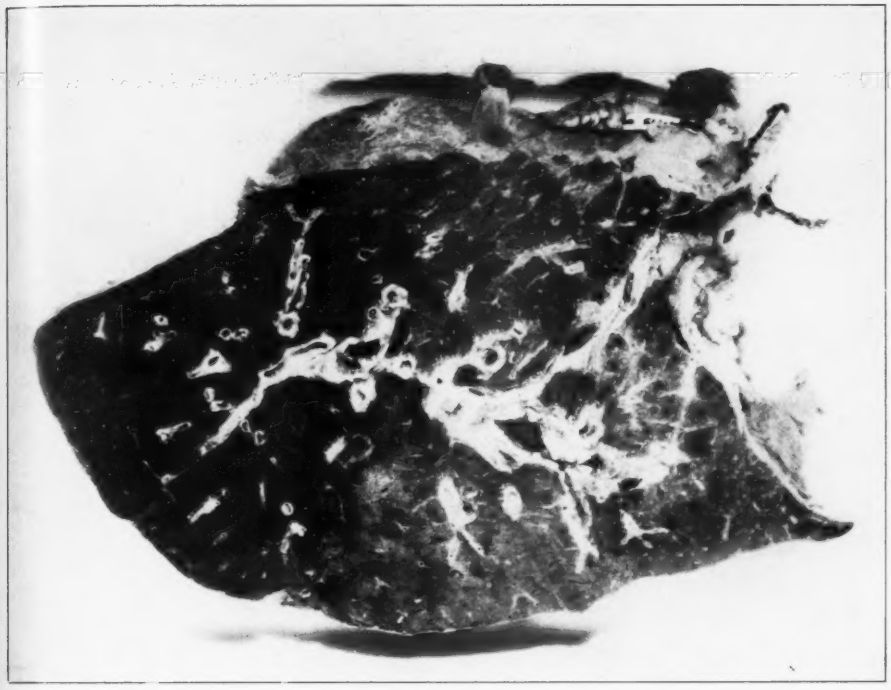
DESCRIPTION OF PLATES

PLATE 106

FIG. 1. Mitral valve from Case 1, viewed from above. Note narrow, slit-like, rigid orifice.

FIG. 2. Lung from Case 1. Upper half red and congested, lower half gray and indurated.



Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

PLATE 107

FIG. 3. Diagrammatic drawing of structure of alveolar wall, emphasizing the location and relations of the basement membranes.

A = epithelial cell lining alveolus
B = alveolar basement membrane
C = capillary basement membrane
D = endothelial cell lining capillary

FIG. 4. Normal alveolar wall. Capillary sufficiently wide to admit passage of a single red cell. Alveolar and capillary basement membranes appear as a single thin line. Aniline blue stain. × 670.

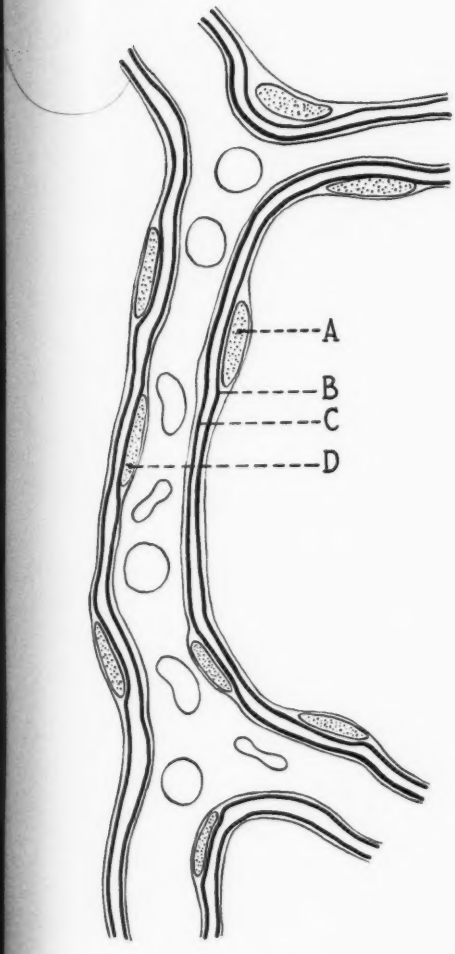


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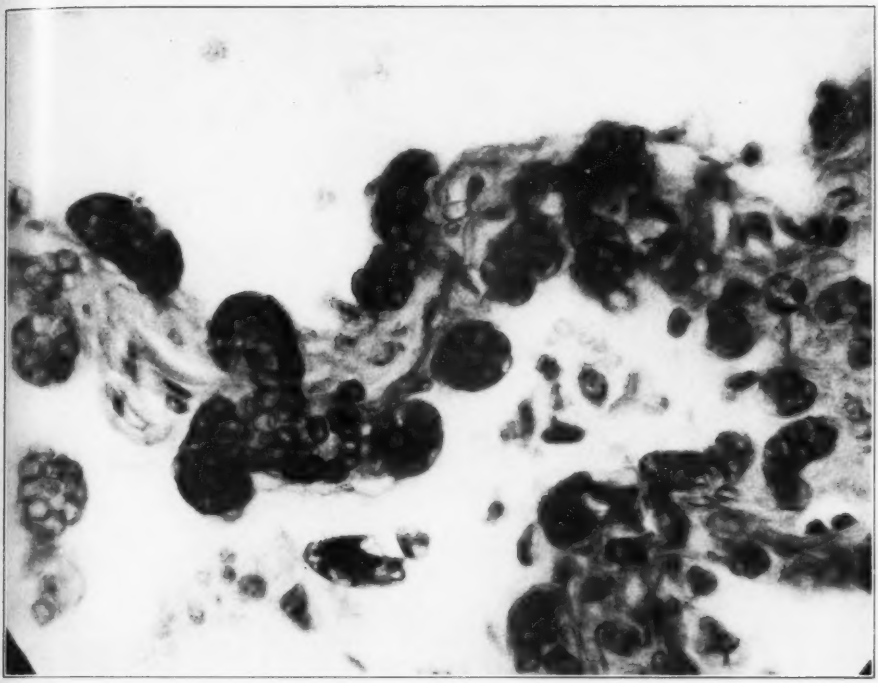
PLATE 108

FIG. 5. Section from tip of apex from Case 1. Congestion and increase in number of visible capillaries, and dilatation of same with herniation into alveolar spaces. Each capillary is sufficiently wide to admit the simultaneous passage of several red cells. Aniline blue stain. × 670.

FIG. 6. A somewhat more advanced stage than that shown in Fig. 5. In addition to the dilatation and increased number of visible capillaries, there is thickening of the capillary basement membrane. Aniline blue stain. × 600.



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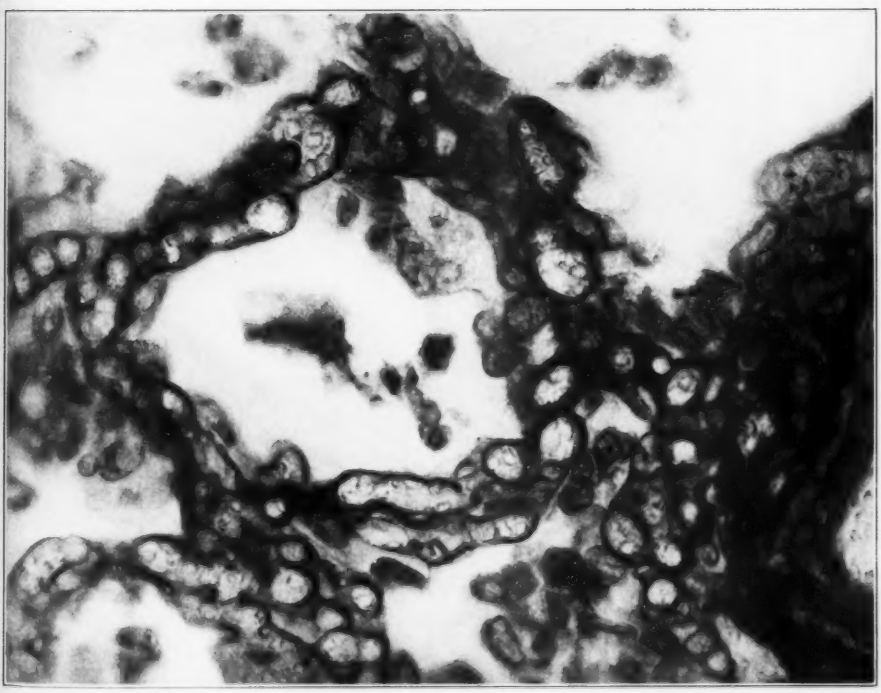

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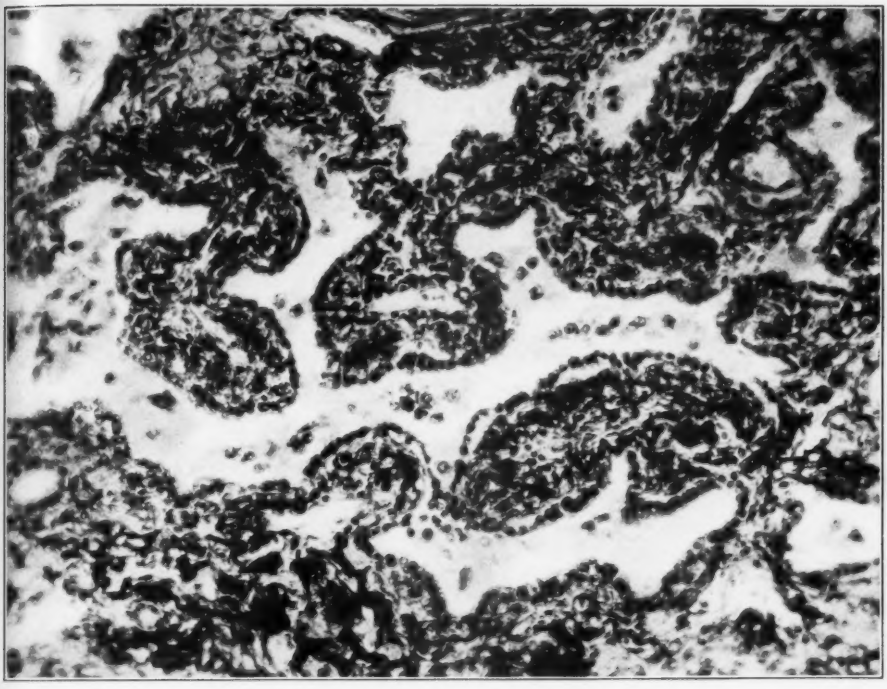
Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

PLATE 109

FIG. 7. Late stage. Alveolar walls thickened and covered with cuboidal cells. Capillaries small and displaced from the alveolar spaces. Aniline blue stain. × 210.

FIG. 8. Pericapillary edema. The thin, unchanged alveolar basement membrane is ballooned out by the fluid. Aniline blue stain. × 670.



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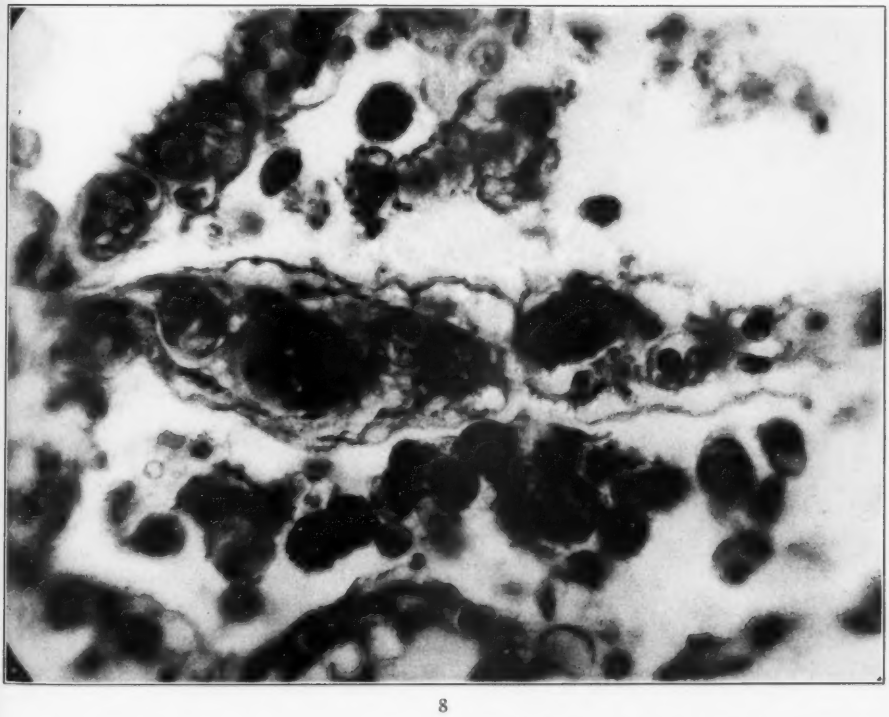

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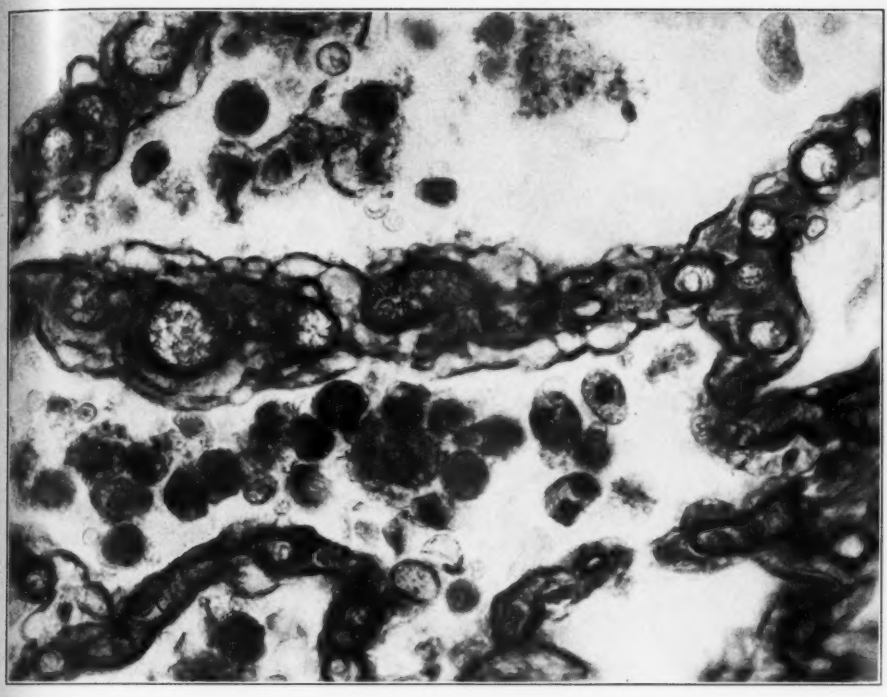
Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

PLATE 110

FIG. 9. Pericapillary edema. Alveolar basement membrane as in Fig. 8. In addition, thickening of capillary basement membrane. Aniline blue stain. × 600.

FIG. 10. Emphysema of a previously congested alveolus as indicated by increased collagen in the wall. Capillary lumens less than normal in diameter. Aniline blue stain. × 285.



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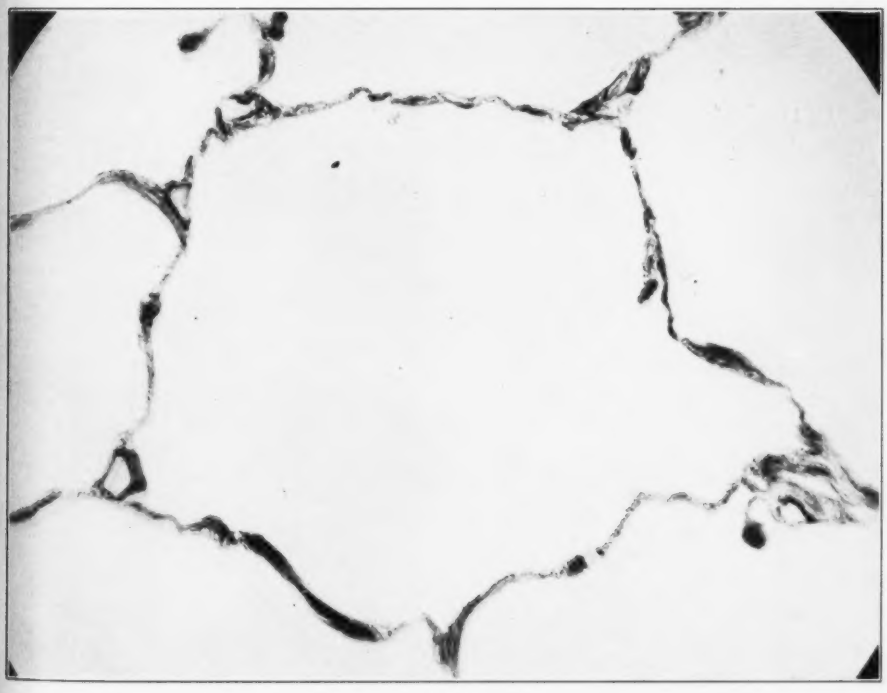

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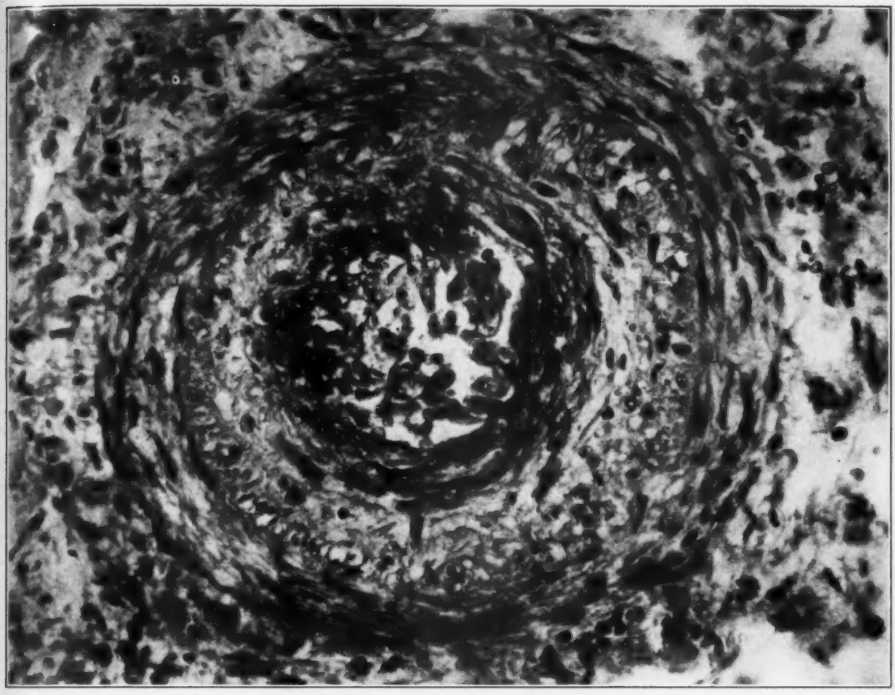
Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

Plate III

Fig. 11. Case 1. Rheumatic arteritis, early stage. Subendothelial deposition of fibrin with proliferation of endothelial cells. Phloxine-methylene blue stain. × 360.

Fig. 12. Case 1. Rheumatic arteritis, late stage. Lumen represented by endothelial lined spaces. Intima consists of vascular connective tissue. Phloxine-methylene blue stain. × 360.



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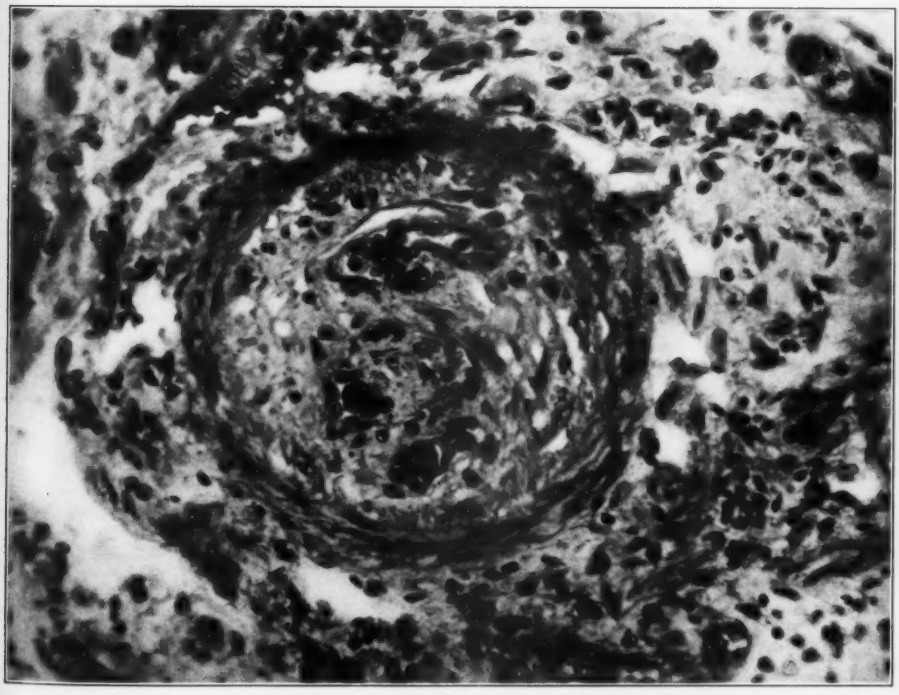

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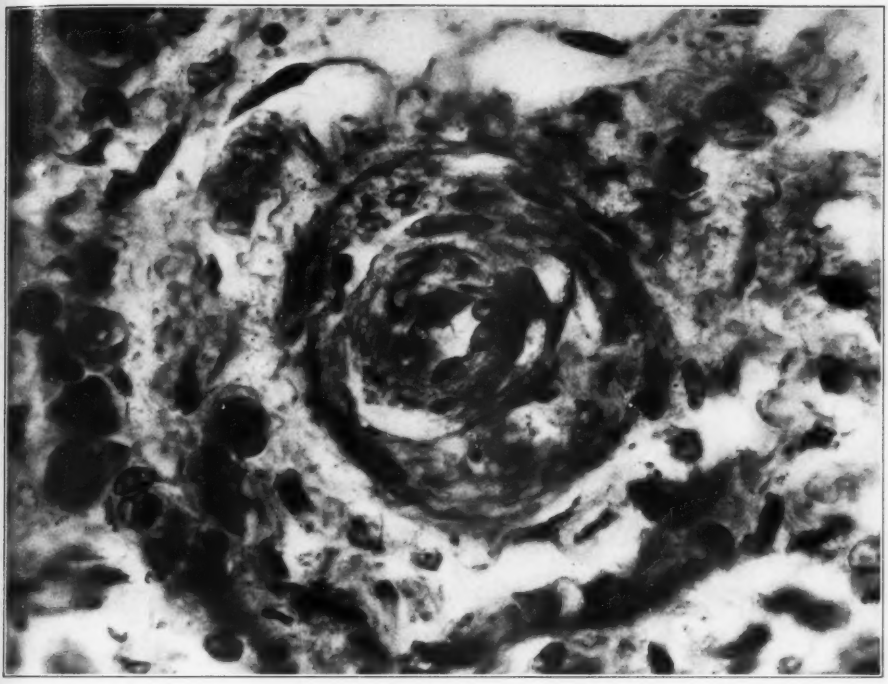
Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

PLATE 112

FIG. 13. Hyperplastic arteriolosclerosis. Phloxine-methylene blue stain. × 800.

FIG. 14. Hyperplastic arteriolosclerosis. Phloxine-methylene blue stain. × 800.



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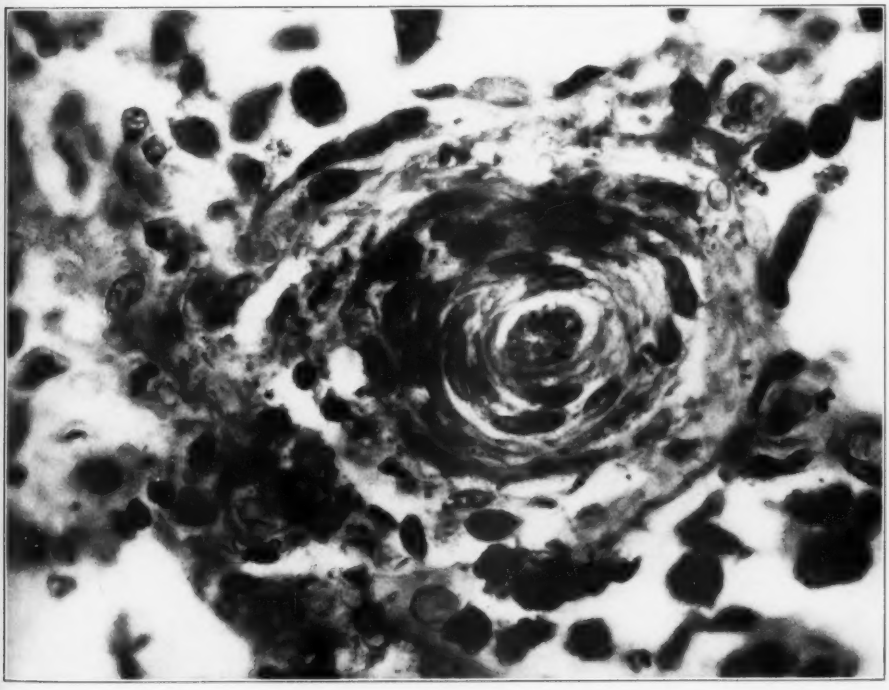

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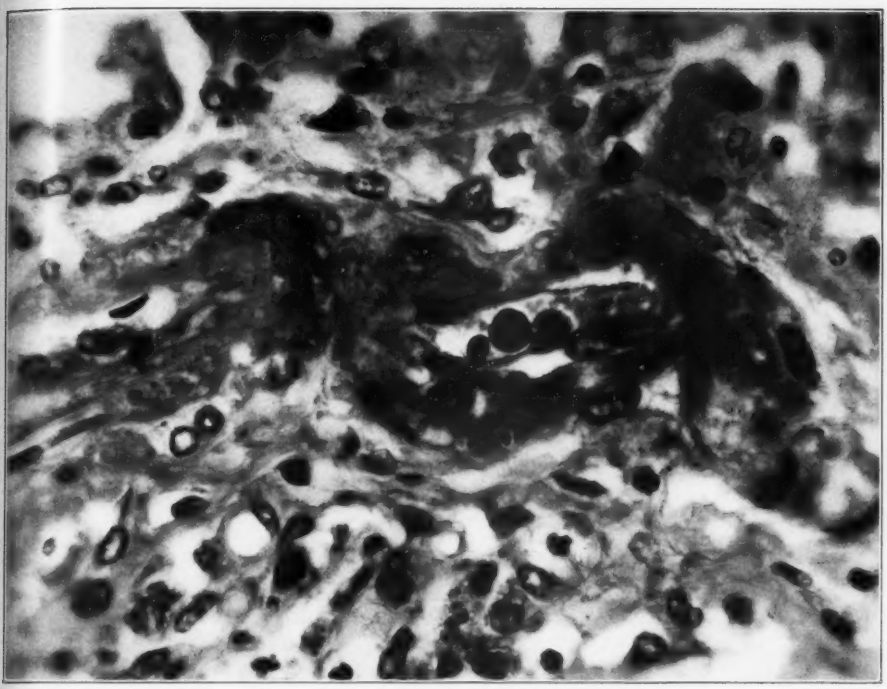
Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

PLATE 113

FIG. 15. Necrotizing arteriolitis. Phloxine-methylene blue stain. × 800.

FIG. 16. Edema of septum with no edema of adjacent alveoli. Aniline blue stain. × 80.



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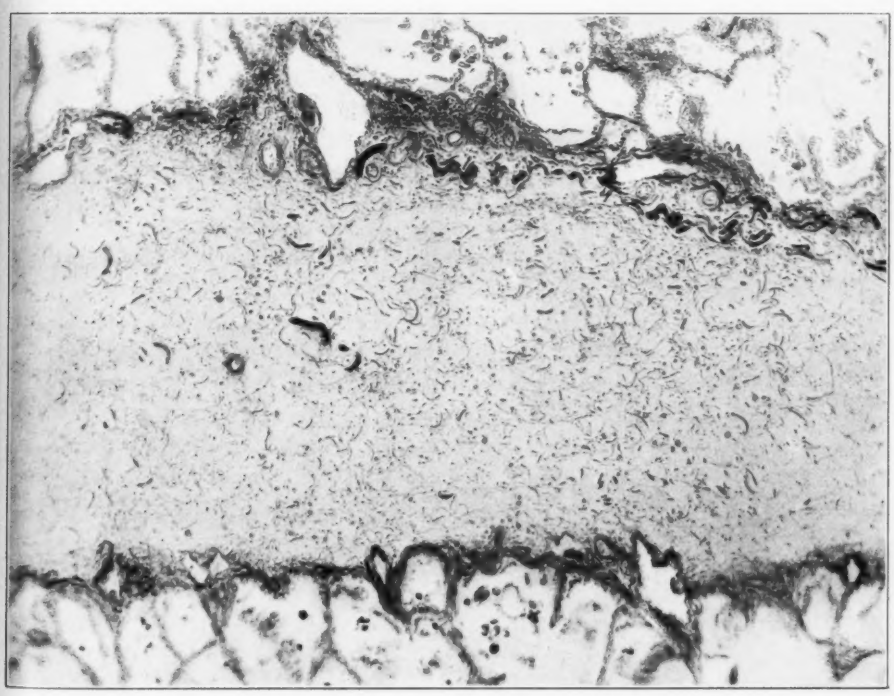

16

Parker and Weiss Structural Changes in Lungs in Mitral Stenosis

THE EVOLUTION AND INVOLUTION OF THE PROSTATE GLAND *

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INTRODUCTION

The earlier literature contains excellent studies on the histology of the prostate by Walker,[1] Langerhans,[2] Moullin,[3] Petersen,[4] Weski,[5] and Pallin.[6] Investigations of the structure of the prostates of animals have also been made by Walker,[7] Stilling,[8] and de Bonis.[9] The entire literature on this subject is reviewed by Macklin.[10] In addition a few investigators, particularly Englisch,[11] have published reports on senile atrophy of the prostate. The surgical aspects of this atrophy are well discussed by Datyner,[12] Dubs,[13] and Shen.[14] In none of these studies is the histological appearance correlated with the age and related prostatic and visceral disease so that etiology and pathogenesis can be determined. The recent investigations on the sex hormones, summarized by Allen and his collaborators,[15] have opened a new mode of approach to these problems. By experiments with partially endocrinectomized animals with controlled parenteral administration of hormones the morphological appearances in man may be reproduced and correlated.

The present study was undertaken originally at the suggestion of Prof. J. Erdheim, in Vienna, as a morphological investigation, and later the experimental aspects were undertaken at Cornell University under Dr. Eugene L. Opie. The morphological studies were carried on and largely completed at Western Reserve University under Dr. Howard T. Karsner. To these three I am indebted for their guidance and support. The National Research Council, through the Medical Fellowship Board and the Committee on Grants-in-Aid, has made possible the technical part of the work.

The basic material for study consisted of 678 prostates secured from consecutive autopsies at the Prosektur of the Krankenhaus der Stadt Wien from August 1931 to July 1932. The prostate was secured at autopsy, left uncut and fixed for 10 days to 6 months in

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10 per cent formalin. After fixation the gland was sectioned sagittally with each section not over 4 mm. in thickness. Paraffin sections stained with hematoxylin and eosin were prepared from each block. The whole blocks were not subdivided unless they were too large for a 3 by 2 inch slide. Additional sections for special stains were prepared as seemed desirable. The Masson trichrome stain with light green, and the Verhoeff elastic tissue stain were especially instructive. In some instances longitudinal and horizontal sections were prepared to illustrate special points. By this step-section method of study every lesion of the prostate larger than 4 mm. in diameter, and many smaller ones, were observed. For the special study of lipids smaller blocks were separated from the whole sections, embedded in 20 per cent gelatin and frozen sections prepared. This method gives assurance that small free masses in the lumens are not lost. Routinely, these sections were stained with Ehrlich's hematoxylin and Sudan III. For special purposes Nile blue sulphate and light green and acid fuchsin were used. Every frozen section was examined with polarized light. One hundred and eighteen prostates were examined in frozen section. The incidence of the various diseases and the statistical analyses are based entirely on these 678 unselected cases. In addition, selected prostates from autopsies at the University Hospitals in Cleveland from August 1932 to July 1933, and at the New York Hospital from August 1933 to June 1935, were studied with routine single sections and a few with step-sections.

Morphological Changes at Puberty

In another paper,[16] the evolution of the prostate through the neonatal period and infancy will be presented. At about 10 years of age there is beginning activity in the prostatic epithelium and between the 12th and 14th years the richly branching alveoli with numerous papillae become evident. In Figures 1 and 2 are shown the general appearance of the prostate just before and after puberty. In a series of white rats* of various ages the same puberal maturation has been observed, but in the rat the transition is a more gradual process and early signs of maturation of the prostatic epithelium may be seen at 40 days of age. The clear vacuole in the epithelial cell of the rat's

* These examples have been selected from a series of 15 animals killed on the 1st, 1st, 3rd, 5th, 7th, 10th, 13th, 16th, 20th, 24th, 28th, 36th, 70th, 70th and 70th days of life. All were from the related parents and were known to be fertile at about the 60th day.

prostate after Bouin's fixation may be observed as early as the 20th day.

The explanation for this puberal prostatic maturation is part of the general problem of the cause of puberty. However, as a working hypothesis the experimental results of hormone injections may be utilized. The rapid maturation of the prostate in 48 to 96 hours, which follows the injection of either the pituitary-like hormone of pregnancy or the male sex hormone, is well known and the results of our few experiments need not be detailed or figured. However, it is clear that the histological changes in the prostate at puberty may be duplicated by the injection of either the male sex hormone or the pituitary-like hormone of pregnancy in mice or rats. This effect of pregnancy urine has been noted by others and the literature is summarized by Bourg.[17] Smith and Engle,[18] and Moore and Price,[19] have described similar enlargement of the accessory sex glands in immature animals after pituitary implants. That the pituitary hormones do not act directly on the prostate is shown by the results of injections in castrated rats and mice. Although hypophysectomy induces changes in the prostate identical with those produced by castration (see Smith [20]), the injection of pituitary extracts into castrated rats will not affect the atrophy of the prostate. From these studies it has been assumed that the pituitary hormone affects the testis, which in turn secretes a hormone that affects the prostate (see Engle [21]). Moore [22] has demonstrated that increasing amounts of testicular hormone depress the secretion of the pituitary hormone. Brouha, Hinglais and Simonnet [23] suggested that this response of the prostate and vesicles to pregnancy urine be used as the basis of a test to replace the Aschheim-Zondek and Friedman test in the female. On the contrary, the effects of the various pituitary extracts and implants on the process of testicular maturation are extremely confused by contradictory reports (see Engle [24]).

Griffiths [25] has described histological changes in the prostate of the hedgehog awakening from hibernation entirely analogous to those which occur at puberty. The alteration in the cellular pattern of the pituitary during hibernation, described by Cushing and Goetsch,[26] is probably a related phenomenon. Womack and Koch [27] could not demonstrate the male sex hormone in the urine of boys under 10 years of age. From the available anatomical, physiological and experimental evidence, the maturation of the prostate at puberty and

in the spring in hibernating animals is apparently the result of an increased quantity of the testicular hormone which reaches the prostatic tissues. The reported investigations at variance with this assumption indicate that there are probably other factors to be considered, such as the release of the hormone by the gland of origin, the renal threshold for the excretion of the hormone, the reactivity of the tissue to the action of the hormone, and possible interreactions with other endocrine glands, particularly the thyroid and adrenal. It is also possible that the extraction methods used by different investigators produce variations in the quantitative and qualitative yield.

Macroscopic Appearance of the Normal Prostate

On both macroscopic and microscopic examination it is possible to divide the structures contained within the capsule of the prostate into several groups: (1) the urethra and the small evaginations from it; (2) the deferential canal, which contains the ejaculatory ducts, prostatic utricle, and at times a part of the seminal vesicles; (3) the periurethral glands; and (4) the prostate proper.

The parts designated as prostate may be further subdivided into the stroma and epithelial-lined ducts and acini. On the basis of the location of the acini and the orifice of the ducts the lobules of the prostate are collected into lobes and designated as posterior, anterior, lateral and middle (Fig. 3). Lowsley [28] has shown that this lobar division has both an anatomical and an embryological basis. The posterior lobe is that group of glands posterior to the urethra and deferential canal, the ducts of which empty into the floor of the urethra caudad to the verumontanum. The anterior lobe comprehends those acini directly anterior to the urethra, the ducts of which empty on the roof of the urethra. The middle lobe is between the urethra and the deferential canal and its ducts are found in the floor of the urethra cephalad to the verumontanum. The lateral lobes are paired, located on the lateral aspect of the urethra and the ducts empty in the furrows on each side of the verumontanum. With the exception of the rare cases in which the middle lobe is absent, these lobes are a constant finding. Early in development the terminal acini become intertwined with one another and there is not a distinct line of separation in any one section.

The periurethral glands, usually divided into a subtrigonal group

and a collicular group, must be considered by the pathologist since numerous investigators have claimed that the glandular tissue in benign enlargement is derived from them and not from the prostatic acini or ducts. The structures of the deferential canal and the urethra are not directly concerned with the scope of this study and will not be discussed.

On sagittal section (Fig. 4) through the center of the gland the typical macroscopic appearance can be demonstrated. The gray or grayish yellow ducts to the lateral lobe radiate from the posterior extremities of the V or inverted Y shaped urethra. The acini appear as light orange-yellow, slightly elevated granules with or without a lobular arrangement. The posterior lobe is directly posterior to the urethra but at its lateral borders cannot be sharply delimited from the lateral lobes. The acini are usually more distinctly orange-yellow or orange in color, in contrast with the purer yellow of the other lobes, and the lumens or even slight depressions in the center of each gross acinus cannot be seen as clearly as in the lateral lobe. The anterior lobe may usually be recognized grossly. The acini are gray or grayish white in color and few in number. The lateral lobe is large and the acini definitely apparent. They are usually gray or grayish yellow but some isolated areas may be orange-yellow. Anterior and medial to the ducts the lobular markings are distinct and lumens can rarely be seen. These glandular groups are usually yellower than the remainder. Lateral to the ducts, lobules cannot be as easily identified and dilatation of the acinar lumens is common, especially just beneath the capsule. The stroma throughout the entire gland is gray or grayish white and opaque, but in the posterior lobe it may be bluish gray and semitranslucent. About the urethra, and separating it from the lateral lobe, there is a definite zone of gray fibrillar tissue which contains only an occasional small gray acinus.

On midlongitudinal section the general appearance of the acini is the same. The posterior lobe can now be sharply delimited by the urethra, deferential canal and posterior capsule. The ducts are yellow or yellowish orange and are directed toward the urethra caudad to the verumontanum. The middle lobe is clearly seen anterior to the deferential canal and posterior to the urethra. The acini, especially in the cephalic part, are deep yellow in color. The ducts empty just cephalad to the verumontanum and are gray in color. Anterior to the middle lobe, in the floor of the urethra, the periurethral glands

can usually be seen as small gray or grayish white dots in the gray fibrillar tissue. The acini of the anterior lobe, if present, are above or just cephalad to the verumontanum in the roof of the urethra.

By exact observations of the gross structure certain deductions may be made concerning the character of the glands and the epithelium. A pure yellow color of the lobules is indicative of tall epithelium and is not related to lipid content; the deeper the yellow color in the gross, the taller the epithelium. A gray color of the lobules is almost constantly associated with a low cuboidal epithelium. In contrast to the pure yellow, the addition of an orange element means the appearance of demonstrable lipid in the epithelial cells. This lipid can be stained with scharlach R and Sudan III and a part of it is doubly refractile. As a confirmatory observation on the character of the epithelium the character of the granulation of the lobules may be employed. A coarsely granular cut surface within each lobule is evidence that the epithelium is thrown into papillary folds, while a finely granular surface is associated with small round acini with cuboidal epithelium and free of papillae. In the ducts the variation in color from gray to yellow is the result of the presence of transitional or columnar epithelium respectively. A pale yellow zone about the larger ducts is due to the concentration of elastic fibrils in this region. The color of the stroma is an admixture of the color of smooth muscle and collagenous connective tissue.

Size of the Prostate at Different Ages

When blocks were cut from the fixed prostates, measurements of the three axes were made in order to secure information on the size in the successive decades. After the sections were prepared, all sections of each prostate were examined for pathological changes other than those which will be discussed later as typical of senile involution. With these criteria there are available measurements on 129 prostates. It is freely acknowledged that there may be some alterations that would affect the size of the gland and that have not been recognized. All cases of benign enlargement, carcinoma and manifest infection have been eliminated, regardless of the size of the lesions. The volume has been calculated on the assumption that the prostate is a flattened prolate spheroid and that the formula

$$\text{Volume} = 4/3\,\pi\,a\,b\,c$$

is applicable when a, b and c are the semiaxes.

TABLE I

Size of Prostate

Decade	Observations*	Volume	Cephalocaudad axis	Lateral axis	Anteroposterior axis
		cm.	*cm.*	*cm.*	*cm.*
3rd	22	10.22 ± 1.09	2.75 ± 0.116	3.60 ± 0.119	1.89 ± 0.076
4th	24	11.72 ± 0.799	2.92 ± 0.111	3.71 ± 0.088	1.95 ± 0.193
5th	18	10.88 ± 0.788	2.87 ± 0.085	3.67 ± 0.083	1.96 ± 0.109
6th	31	12.03 ± 0.804	2.91 ± 0.071	3.75 ± 0.086	2.06 ± 0.076
7th	22	12.06 ± 1.01	2.85 ± 0.114	3.68 ± 0.085	2.05 ± 0.087
8th	9	11.94 ± 1.11	2.87 ± 0.080	3.78 ± 0.125	2.05 ± 0.115
9th	3	13.70 ± 1.39	3.00 ± 0.205	4.37 ± 0.173	2.17 ± 0.238

* The 120 individual observations have been studied by the statistical method of analysis by variance for significant differences between the decades.

TABLE II

Analysis of Variance of Volume of the Prostate in Different Decades

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	128	2,351.00	18.36
Between decades	6	74.67	12.44
Within decades	122	2,276.33	18.65

F 1.49

TABLE III

Analysis of Variance of Cephalocaudad Dimension of the Prostate in Different Decades

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	128	26.87	0.21
Between decades	6	0.50	0.08
Within decades	122	26.37	0.22

F 2.75

TABLE IV

Analysis of Variance of Lateral Dimension of the Prostate in Different Decades

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	128	26.14	0.20
Between decades	6	1.66	0.28
Within decades	122	24.48	0.20

F 1.40

TABLE V

Analysis of Variance of Anteroposterior Dimension of the Prostate in Different Decades

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	128	29.17	0.23
Between decades	6	0.59	0.10
Within decades	122	28.58	0.23

F 2.30

TABLE VI

Case No.	Estimated age	Actual age	Difference	Case No.	Estimated age	Actual age	Difference
	yrs.	*yrs.*	*yrs.*		*yrs.*	*yrs.*	*yrs.*
Syphilis				*Diabetes*			
328	65	62	+ 3	177	58	71	−13
353	60	62	− 2	240	55	66	−11
354	63	51	+12	294	60	59	+ 1
401	53	57	− 4	321	65	52	+13
408	75	72	+ 3	347	63	67	− 4
446	65	68	− 3	511	53	57	− 4
466	68	70	− 2	529	62	57	+ 5
478	70	73	− 3	*Leukemia*			
482	55	71	−16	378	70	65	+ 5
493	62	74	−12	418	60	74	−14
498	55	54	+ 1	257	30	39	− 9
546	60	54	+ 6	*Chronic Nephritis*			
563	60	58	+ 2	405	55	63	− 8
575	62	69	− 7	277	50	55	− 5
597	65	61	+ 4	93	25	18	+ 7
Rheumatic Heart Disease				*Hypertension*			
125	40	40	0	277	57	55	+ 2
131	60	65	− 5	332	60	58	+ 2
222	65	74	− 9	360	60	66	− 6
232	65	68	− 3	489	65	70	− 5
272	70	63	+ 7	493	70	74	− 4
302	62	74	−12	496	62	62	0
376	35	43	− 8	501	65	66	− 1
387	40	32	+ 8	513	68	63	+ 5
416	60	60	0	*Carcinoma*			
Streptococcic Infections				264	57	59	− 2
377	50	55	− 5	266	72	67	+ 5
479	60	58	+ 2	273	62	54	+ 8
563	65	58	+ 7	286	60	50	+10
Multiple Sclerosis				292	72	70	+ 2
272	68	63	+ 5	297	70	70	0
296	60	50	+10	335	40	45	− 5
361	35	24	+11	342	70	71	− 1
391	55	39	+16	362	60	64	− 4
512	58	62	− 4				

In these analyses of variance, F is equal to the greater mean square divided by the smaller mean square and when the observations of the former are infinite and of the latter 7 the least significant F is 3.23. In the reverse, the F must exceed 2.17 to be considered as showing a greater difference between the decades than within the decades. Therefore, we may conclude that on the basis of 129 observations there is no significant difference in the size of the prostate between 20 and 90 years of age.

Microscopic Appearance of the Postpuberal Prostate

Microscopic examination of a midsagittal section similar to that described above reveals the picture shown in Figure 5. This section is taken just cephalad to the orifice of the ejaculatory ducts so that acini of the middle, posterior and lateral lobes are shown. The middle lobe acini anterior and immediately lateral to the deferential canal are sharply separated from the lateral and posterior lobe by stromal septa. The septal markings between the posterior and lateral lobes are not definite. The general lobular architecture is well shown in all parts of the gland and even with this magnification the rich papillary folding of the epithelium is evident and the papillae are equally abundant in all parts, a point that is important as a criterion of adult life. The lobular architecture both grossly and microscopically should be sharply differentiated from the nodular architecture of benign enlargement.

The epithelial cells of the prostate during the 3rd and 4th decades vary considerably in size and structure. In this study an attempt has been made to classify these cells and arrange them in a hypothetical cycle of secretion.

The first type (Fig. 6) is a low columnar or cuboidal cell with a round, moderately chromatic nucleus situated in the base of the cell but not in contact with the cell wall. The nucleus may be of such a size as to occupy two-thirds of the cell, although it usually fills about one-half. The cytoplasm, with formalin or Zenker fixation, is palely acidophilic, slightly reticulated and free from granules. Beneath the cuboidal cell there is an irregular layer of basal cells that can be clearly distinguished as distinct from the surrounding stroma. The nuclei of these basal cells are elliptical with the long axis in the plane of the wall of the acinus. The cytoplasm is inconspicuous but in gen-

eral is slightly denser than that of the luminal cells. The luminal cells vary from 8 to 12 microns in width and each cell is a distinct entity with no pseudostratification of cells or nuclei. The luminal cell wall is well defined and may be either smooth or slightly scalloped on account of bulging of the cytoplasm of each cell. When viewed from the lumen such a group of cells as described would present slight mammillation. This cell varies from 10 to 15 microns in height.

The second type (Fig. 7) is a much taller cell with a slightly smaller, round nucleus. The cytoplasm is essentially the same as that of Type 1 but the cell is only 7 to 10 microns in width. In height it varies from 15 to 20 microns and the cell walls including those toward the lumen are distinct. The basal cells are generally similar to those of Type 1.

The third type presents radical differences from those above described (Fig. 8). The cell is from 18 to 25 microns in height and 9 to 12 microns in width. The basal and lateral cell walls are distinct but the luminal membrane is indistinct or may be entirely lacking, so that it appears that the cytoplasm of the cell is directly continuous with secretion within the lumen (Fig. 9). When there is a remnant of the luminal cell wall the nucleus is elliptical in shape with the long axis parallel to the wall of the acinus, but in those cells where there is rupture the nucleus is round or at the most only slightly ovoid. When the nucleus is elliptical a basal layer of cells cannot be demonstrated but if the nuclei are round the basal cells are as distinct as in Types 1 and 2. The cytoplasm is considerably denser and more acidophilic than that of the earlier described types and when the luminal membrane is intact the cytoplasm is clearly denser in a zone of 1 to 3 microns beneath it. In this denser luminal cytoplasm and extending down into the clearer cytoplasm there are small, variously sized granules which are for the most part acidophilic, but an occasional one is basophilic. The lumens bounded by the epithelium of this type invariably contain granular acidophilic débris which we have considered as prostatic secretion.

The fourth type (Fig. 10) is materially different from any of the previous three types. The nuclei are elliptical with the long axis perpendicular to the wall of the acinus. The chromatin is much denser and basophilic and an internal chromatin network can be demonstrated only with difficulty. The nuclei are closely opposed to

one another with pseudostratification. The cytoplasm is densely homogeneous and more acidophilic than that of the other types. Cell walls are not apparent but the luminal boundary of the cell is smooth and distinct.

The only remaining cell type of the prostatic acini and ducts is the transitional epithelium which is frequently found in the major ducts near the urethra. It conforms entirely to this type of epithelium described in textbooks and need not be considered in detail here.

These various types of epithelial cells and cells that appear to be transitional stages between them may be found in one prostate, but in general there is a tendency for one type to be present in the greater number of acini. Within one acinus two or more types may be found, but again the greater number of cells are of one type.

These cell types are found on the walls of the acini and over the papillae although what is apparently the same cell type is taller and thinner on the sides and tips of the papillae than on the wall. The nuclei are in general more chromatic near the tips of the papillae.

Many of the papillae of the acini in a prostate from an adult male, when studied in serial section, are apparently a phenomenon of local growth. With the increase in the cytoplasmic content of each cell some of the increased pressure is released toward the lumen and manifested as an increase in height of the cell and a bulging of the luminal cell wall, but at some point the pressure is greater and the cells buckle into a small mound without a stromal core. The nuclei in these areas are elliptical with the long axis at right angles to the wall and each cell is very narrow. Other, usually larger, papillae contain a connective tissue centrum with an occasional smooth muscle fiber.

The stroma of the prostate is composed of three essential elements — collagenous connective tissue, smooth muscle fibers and elastic fibers. The arrangement of these elements about the individual acini and the connections with the musculature of the bladder and ejaculatory canal have been studied by Walker,[1] Lowsley,[28] and others, and the possible relation of this anatomical structure to the physiology of urination and ejaculation adequately discussed.

The stroma may be divided into classes, dependent on the location, into periductal, interlobular, intralobular or interacinar, perivascular and perineurial types.

About the larger ducts there are bundles of smooth muscle fibers

which are arranged both longitudinally and circularly or spirally. The former are fine fibers and are arranged in small bundles while the latter are gathered together in larger bundles. Between the muscle fibers in both instances there is a moderate amount of collagenous connective tissue and elastic fibers. The longitudinal elastic fibers are more prominent.

About all the larger and medium sized vessels and nerves there is a layer of pure fibro-elastic connective tissue without smooth muscle fibers. The vessels of arteriolar size and smaller ramify directly in the interlobular and interacinar stroma.

The stroma between and in the lobules is essentially the same although the muscle fibers are more abundant and somewhat larger in the interlobular septa. About the acini there is a definite disposition of the stroma which is of the greatest importance in the proper diagnosis of pathological changes. Immediately external to the cell wall of the epithelial cells there is a thin zone of pure collagenous connective tissue and a few elastic fibrils. In this layer are long, narrow, at times wrinkled nuclei — presumably fibrocytic nuclei. A few of the capillaries are found between these collagenous fibrils but an endothelial lined channel has never been observed in direct contact with epithelium. Outside this layer is stroma composed of approximately equal parts of smooth muscle and connective tissue arranged in sweeping arcs about the acini in such a manner that contraction of the muscle would result in reduction of the size of the lumen from all sides equally.

Morphology of the Process of Prostatic Secretion

The difficulties of a study of a secretion process, on the basis of isolated morphological observations on autopsy specimens, are apparent. In the evidence which has been presented it would appear that there is a cycle in the epithelial cells. The small cuboidal cell is the type of cell that is designated as a "resting cell" in other glands where secretion has been studied. In contrast is the tall columnar cell of Type 3 with the cytoplasm continuous with secretion in the lumen, a process of apocrine * secretion that has been described in other glands. Type 4 is definitely a cell of hyperplasia with crowding

* The term apocrine is used here to designate a process in which a portion of the cytoplasm of a cell is discharged as secretion. When the entire cell is converted into secretion the term holocrine is applied to it.

of the nuclei and an increase in the intensity of staining. In order to secure a working hypothesis the cell types have been ranged in a drawing (Fig. 11) in the order these observations indicate is correct. There is no related change in the stroma as the cells increase in height and discharge a portion of their cytoplasm.

Morphology of the Presenile Prostate

Manifestly, any classification of a gradual process is artificial and therefore no attempt will be made to describe the morphology of the prostate during each decade after 40 years. However, it would appear that the involution proceeds with greater rapidity during the 5th and 6th decades and that after 60 years the changes are less striking and the velocity is greatly decreased. Therefore, the process has been divided into a presenile period and a senile period with the dividing line at approximately the 60th year of life. In any one individual or when only one morphological criterion is employed, the age of onset of definite senility will vary, but on the whole, old age, as determined by the morphology of the prostate, may be considered as definite at 60 years.

The outstanding characteristic of the presenile gland is the variation in the appearance of the same structure in different parts of the same prostate, in contrast with the uniform appearance of the adult prostate.

In the stroma there is a gradual atrophy of the smooth muscle fibers and relative or perhaps absolute increase in connective tissue. The cells of the connective tissue retain their adult characteristics and there is no histological evidence of proliferation. The nuclei are small, highly chromatic and with increasing age may show irregularity in outline and even pyknosis. The collagen fibrils become denser and agglutinated, although clear hyalinization is rare in the presenile period. There is an increase of the collagenous tissue about each acinus so that the layer, which in adult life is 5 to 15 microns in thickness, is expanded to a layer 20 to 50 microns. The smooth muscle fibers are smaller but pigmentation is not a consistent part of the picture. When pigmentation of these fibers is found the granules are similar to those found in other organs with atrophy and senility.

The acini at this period are in general larger and the papillae are not as numerous, but a not inconsiderable number of alveoli are

lined by an epithelial layer essentially the same as in the earlier decades. The hyperplastic cells described as Type 4 are more conspicuous and pseudostratification is more apparent (Fig. 10). The nuclei are more chromatic and the cytoplasm more acidophilic and denser, that is, the morphological criteria of hyperplastic cells. The majority of papillae that are present are of the same two types described in the adult prostate, but in addition there is another type observed at this period. The cells are thrown into a pedunculated spherical mass without a connective tissue centrum and in the center of such a mass there is not infrequently a lumen. Thus, there is formed an acinus with a lumen within the epithelium of the wall of a larger acinus. The individual epithelial cells in these pseudo-acini are deeply acidophilic and denser and the nuclei are larger and more chromatic than the cells of the adjacent wall. A nucleolus is not conspicuous and the shape of the cell varies from polygonal to columnar with or without orientation as regards the major or minor acinus.

Metaplasia may occur during the presenile period. There is a focal area, usually in the ducts but occasionally in the terminal alveoli, where the usual epithelium is replaced by a mass of spindle cells with general orientation of the long axis at right angles to the wall of acinus. The luminal cells are more cuboidal and definitely oriented toward the lumen. A basal layer or even a basement membrane is lacking but there is no evidence of invasion of the stroma. This is a solitary alteration without associated change in the stroma and without surrounding inflammatory infiltration.

In contrast with these manifestations of cellular growth, namely hyperplasia, pseudo-acinar formation and metaplasia, atrophy is a conspicuous feature of the presenile period. This atrophy may involve only the epithelial cells or the acinus as a complete structure, or the two types may be combined.

Atrophy of the epithelium results in a decrease in the height of the cell and all stages may be observed. As landmarks two types have been selected and designated as Types 5 and 6. In Type 5 (Fig. 12) the cells are in one layer and vary from 8 to 11 microns in height and 10 to 13 microns in width. The cytoplasm is granular or reticulated and a round nucleus fills at least one-half of the cell. The cell walls are distinct and the membrane toward the lumen is smooth and without the scallops seen in earlier decades in Type 1. There is no evidence of secretion.

The cell that has been designated as Type 6 (Fig. 13) is still flatter and rarely exceeds 8 microns in height. The nuclei are round or slightly flattened and are equal in diameter to the height of the cell. In a section 7 to 10 microns in thickness the nuclei overlap one another but are not stratified. The cytoplasm is homogeneous and acidophilic. The luminal cell membranes are sharp and smooth and without evidence of secretion. Rarely the nucleus may be larger than the cell so that it bulges into the lumen or into the surrounding stroma. This cellular type is the highest grade of simple epithelial atrophy that has been observed at any age and it is not seen as frequently in the presenile as in the senile period.

Associated with the atrophy of the individual epithelial cells are changes in the size and shape of the acini and in the surrounding stroma. These changes are of two types which have been designated as simple acinar atrophy and sclerotic atrophy.

Simple acinar atrophy (Fig. 14) usually involves an entire lobule, although isolated acini may be affected. The acini are small, closely packed together and lined by epithelial cells of Type 5 or 6, but some acini or individual cells may be taller and show evidence of secretion. Many of the lumens contain a granular acidophilic material similar to secretion. The surrounding stroma shows no fibrosis and the muscle fibers are abundant and whorl about the acini in the normal manner. The collagenous basement membrane is only slightly thickened.

Sclerotic atrophy is a much more complex process. The earliest lesions would appear to be a simultaneous atrophy of the epithelium and a proliferation of the fibroblasts immediately about the acinus. This proliferation may be regular so that the lumen is compressed equally and appears in section as a round or slit-like structure. It may, however, be irregular and result in distortion of the lumen (Fig. 15). Continued proliferation results in hyalinization of the collagen which is thrown in folds that follow the configuration of the original acinus. The epithelium becomes extremely flattened (Fig. 16) and eventually the sides meet and the epithelium can no longer be identified. The most centrally placed fibroblasts of the collagenous collar are loose and mesenchymal-like so that when the epithelium is lost the center of the former acinus is occupied by a loose tissue of stellate and fusiform cells without collagen (Fig. 17). With continued maturation of the central fibroblasts the whole is

converted into a small hyaline mass which is recognized with difficulty and probably eventually becomes assimilated into the stroma and cannot be demonstrated. There is at no time any exudative inflammatory reaction. Sclerotic atrophy may involve large areas, usually just beneath the capsule and in the posterior lobe, or single ducts or acini may show the lesion. The changes appear to be similar to those described by von Recklinghausen [29] in Bartholin's gland as *myxangioitis hyalinosa*.

The etiological factor in the production of sclerotic atrophy is not clear. It is frequently associated with arteriolar sclerosis and it is possible that the change is analogous to the atrophy and fibrosis that occur in the heart, kidney and other organs as the result of vascular disease. Since this vascular thickening is not associated with generalized arteriosclerotic disease and hypertension, it must be concluded that it represents an involutionary sclerosis similar to that which occurs in the uterus after the menopause.

Morphology of the Senile Prostate

In contrast with the presenile prostate with irregularity of structure and gradual progression of changes the senile prostate represents a more static picture or at the most a very slow progression. There are no additional microscopic features which need be described. The atrophy of the epithelial cells and sclerotic atrophy of the acini become more marked so that at 80 years one-half of the acini are usually obliterated and all of the remaining cells are low cuboidal or flat and there is no evidence of secretion in any alveoli.

A midsagittal section of the prostate in the senile period shows clear differences from that of the young adult described in the previous paragraph. The cut surface is composed largely of stroma with discrete white or gray, rarely yellow granules in which there are many light brown or black corpora amylacea. The stroma in many places is pale blue and translucent. There are few dilated acini and very little fluid can be expressed from the surface. There may be focal areas of orange-yellow or orange acini which contain quantities of doubly refractile lipoid.

The only remaining feature of the senile prostate is the presence of corpora amylacea, but since a separate paper [30] has been devoted to them it is not desirable to enter into the subject at this time.

Estimation of Age

On the basis of the morphological observations, which have been recorded above, it should be possible to estimate the age of an individual by a microscopic examination of a section of the prostate. This has been done on 62 prostates (Table VI) and the errors are shown in Table VII.

Table VII

Estimate of Age on Basis of Morphology

Amount of error	Age less than 74 years		Age above 75 years	
0 to 5 yrs.	58.9%	84.9%	11.1%	22.2%
6 to 10 yrs.	26.0%		11.1%	
11 to 15 yrs.	12.3%	15.1%	38.9%	77.8%
16 to 20 yrs.	2.8%		27.8%	
Above 21 yrs.	0.0%		11.1%	

The zero order correlation coefficient of estimated and actual age is $+0.61 \pm 0.07$. It is apparent that the accuracy is high if the patient is less than 74 years old and that increasing age above this point results in increasing error. It has been assumed from this that the involutionary process reaches a maximum point at about this age and does not proceed further. That the morphological changes are sufficiently constant to allow of an estimate of this accuracy furnishes strong support to the conception of physiological senile involution. If the process were some pathological change or definite disease the correlation of estimated and actual age would not be so close. When an alteration of structure proceeds at a predictable rate in 85 per cent of individuals it cannot be called a disease but must be regarded as physiological and inevitable. Table VI also demonstrates that the diseases shown have no material effect on the process of involution.

The criteria on which these estimates of age have been made may be summarized as follows:

(*a*) Slight irregularity in the height of the epithelium begins between 40 and 45 years.

(*b*) Lobular atrophy begins between 45 and 50 years.

(*c*) The glandular epithelium loses its secretory activity between 50 and 60 years.

(*d*) Sclerotic atrophy first appears between 60 and 65 years.

(*e*) Atrophy of smooth muscle and relative or absolute increase of the fibrous tissue of the stroma is first apparent between 60 and 70 years.

(*f*) Laminated corpora amylacea increase in number and size after 65 years.

Thus, on the basis of microscopic structure of the prostate the life of man may be divided into several periods: first, fetal period, from the first appearance of the prostate at about the 3rd fetal month to a few weeks before birth; second, neonatal period, an interval of 2 to 3 weeks just before and after birth at term; third, childhood period, from 2 to 3 weeks after birth to 10 years; fourth, a prepuberal period, from 10 to 13 years; fifth, an adult period, from 13 years to about 45 years; sixth, a presenile period, from 45 to about 55 years; seventh, a senile period, from 55 years to 75 years; and eighth, a completely senile period, after 75 years.

As would be expected in any biological organism, every example does not fulfill these criteria. In Table VI of the estimated and actual ages of 91 prostates there is a deviation of greater than 11 years in 15 per cent of cases when the age is less than 70 years. These deviations may be divided into three classes: (1) those in which the age was overestimated under the term pathological involution; (2) those in which the age was underestimated under the term delayed senility; and (3) a group of cases in which the age may be correctly estimated but the appearance is not that which has been described for the actual age. The morphological picture of the latter is one of tall columnar epithelium in acini which show all the other characteristics of atrophy. This condition has been designated as secondary hyperplasia and may be diffuse or focal in character.

Pathological Involution

As in the previous morphological observations of senile involution, the changes of pathological involution may be divided into three groups: (1) those in the epithelium, (2) those in the stroma, and (3) the deposition of corpora amylacea. The epithelial alterations consist of a decrease in height and width of the cell, and a change in the character of the nucleus and cytoplasm. The cell, as contrasted with the finely reticulated tall columnar normal type, is low colum-

nar or cuboidal with a dense, usually acidophilic, rarely lightly basophilic cytoplasm. The nucleus is smaller and chromatic with little evidence of a chromatin thread and occupies the greater part of the cell (Fig. 18). In the usual paraffin sections there is frequently overlapping of the nuclei because the decreased diameter results in a section more than one cell in thickness. The luminal cell border is sharp and there is no evidence of secretion. The lumens are empty or contain a few partially degenerated desquamated cells. With Sudan III or polarized light there is no lipid present within the cells or lumens. The acinar atrophy has never been observed to go on to sclerotic atrophy and complete loss of acini as in the senile type.

As contrasted with senile involution the changes in the stroma are inconspicuous. This is probably because of the average short duration of the involutionary process. In the former the epithelial changes appear during the 5th decade while stromal alterations are first manifest early in the 7th decade, a difference of 15 to 20 years. Thus it may be assumed that those systemic lesions that cause involution of the adult prostate do not last long enough to bring about stromal fibrosis. At the most there is a slight relative increase of the connective tissue and decrease of the smooth muscle.

Corpora amylacea are characteristic of the involuting and involuted gland. Small corpora are found in about 25 per cent of glands from individuals between 20 and 40 years of age (in this series 25 per cent between 21 and 30 years and 21 per cent between 31 and 40 years). These corpora are associated with some atrophy of the epithelium and are usually from cases of tuberculosis or other chronic infections. In all these cases the corpora differ in general configuration from those in senile prostates. There are few concentric rings, facets are rare, central pigmentation is unusual and calcification has not been observed. Central softening occurs more frequently than in the senile gland. Many corpora are homogeneous or finely granular without internal architecture. These findings indicate that the typical corpus amylaceum in the senile prostate is the product of several months or years of growth and that the concentric lines are layers of successive deposits. In the young man with pathological involution there has been only a short period of deposition without intervening periods of non-deposition. When concentric lines occur they are less distinct, further apart, frequently wavy, and the layers are less compact. These characteristics are evidence of softer con-

sistence and less secondary organization and dehydration — again criteria of recent origin.

Thus, the histological appearance of pathological involution may be distinguished from that of senile involution on qualitative grounds. The epithelial cells undergo a similar change but complete sclerotic atrophy does not occur and the stromal fibrosis is most inconspicuous. Furthermore, the corpora amylacea exhibit differences in architecture interpreted as evidence of recent origin.

Delayed Senility

In this category have been included not only any case in which the age was more than 45 years and which showed a completely preserved adult prostate (in all sections), but also any case where the age was over 55 years and where an objective estimate of age placed it at more than 10 years less than the actual age. The incidence of delayed senility and secondary hyperplasia is shown in Table VIII.

Table VIII

Incidence of Delayed Senility and Secondary Hyperplasia

Decade	Total cases	Delayed senility		Secondary hyperplasia	
		Cases	Per cent	Cases	Per cent
41–50	55	17	31	0	0
51–60	65	11	17	3	5
61–70	77	4	5	20	26
71–80	63	0	0	23	37

The oldest case (which showed no epithelial atrophy, no fibrosis of the stroma, and no conspicuous formation of corpora amylacea) was a male, 68 years of age. Microscopic observations on the testes and pituitary gland are not available on the Vienna series and there is nothing in the autopsy protocols that might serve as an explanation of the failure of involution.

The morphological picture of these prostates need not be described since it is identical with that described earlier as the normal adult postpuberal prostate.

Secondary Hyperplasia

The incidence of this histological change given in Table VIII shows definitely that it is associated with senility and occurs in inverse ratio to the picture described as delayed senility.

The histological picture is entirely characteristic. The acini are separated by relatively broad bands of intralobular stroma in contrast with the fine delicate bands in the normal prostate. The shape is distinctly elongated, such as has been described in the senile prostate as slit-acini. There is a relative decrease of smooth muscle fibers and increase of connective tissue of the stroma. These changes in the stroma give the impression that each acinus is separate and distinct (Fig. 19) in comparison to the richly branching, folded, intercommunicating acini of the adult prostate.

In spite of these very apparent characteristics of acinar and stromal atrophy, the epithelial cells are tall columnar. The cytoplasm is reticulated and the internal cell membrane is irregular. Secretion is found in the lumens, but few corpora amylacea. The nuclei are relatively large and vesiculated. The epithelium is thrown into numerous papillary folds but in contrast with the normal, the papillae are largely epithelial cells with only a very delicate connective tissue centrum. In localized areas the cells are of the Type 4 described above, that is, tall, narrow columnar with a dense acidophilic cytoplasm and elongated chromatic nuclei.

This secondary hyperplasia is always irregular. Some acini show taller epithelium than others, while in a few cases it is definitely focal, either a lobule or a part of the lobule only showing the change (Fig. 20). The hyperplasia in this focus may be toward the secretory type of cell or the tall, slightly anaplastic Type 4 cell.

There is in none of these types of secondary hyperplasia any reaction of the stroma, any multiplication or branching of acini and no suggestion of the formation of nodules. It is observed most commonly in the posterior lobe. The lesion is distinctly one of cellular hyperplasia of epithelium in otherwise atrophic acini.

These morphological observations indicate that during presenility and senility the prostatic tissues may be stimulated and that their response is not even, but some areas react to a greater degree or involute to a lesser degree after stimulation.

Involution of Castration

In 1 case of the Vienna series (No. 602) a 39 year old male had submitted to a bilateral orchidectomy 5 years before death because of homosexuality. He and the surgeon realized that the operation would probably be of no value but he was anxious to secure any relief from this inversion. He died of chronic pulmonary tuberculosis.

Grossly the prostate measured 2.6 by 3 by 1.2 cm., as compared to 2.9 by 3.7 by 1.9 cm. as the average for this decade (see Table I). It was firm in consistence and on section only a few gray acini could be seen. In a few areas there was dense, fibrillar white connective tissue. The seminal vesicles were exceedingly small.

Microscopically there was an increase of connective tissue in the stroma and a decrease of smooth muscle. There was no pigmentation of the smooth muscle fibers. The acini were small, widely separated and slit-like with the long axis in the peripherohilar direction. The epithelial cells were low cuboidal or at times flattened with relatively small chromatic nuclei which occupied the greater part of the cell (Fig. 23). The marked decrease in cellular size was well illustrated by the nuclear overlapping in ordinary sections.

Physiological Correlation

From the morphological observations described in the preceding sections it would appear that the physiological stimulus necessary for the maintenance of the normal prostate appears at puberty and after a period of 30 years gradually decreases for another 25 years and then is entirely absent. The histological picture of secondary hyperplasia further indicates that this stimulus or the reactivity of the prostate tissues is irregular during the late presenile period.

In all mammals that have been studied, the removal of the testis brings about an atrophy of the prostate. The general character of the changes is similar to those that have been described in senile involution in man. The appearance of the prostate in a senile male (Fig. 21), a senile rat (Fig. 22), a castrated male (Fig. 23), and a castrated rat (Fig. 24), are shown. In all of these there is a decrease in the height of the epithelium, loss of papillae, and prominence of the periacinar collar of stroma. This correlation is further supported when the prostate is examined for long periods after castration.

After 120 to 150 days a process similar to sclerotic atrophy appears, as shown in Figure 25. There is the same collar of hyalinized, relatively acellular connective tissue and irregularity in the outline of the acinus. If castrated animals are given sufficient quantities of extracts of testis [31] or of male urine [32] the normal appearance may be restored, proof that the atrophy is due to the withdrawal of one or more hormones contained in these extracts.

As shown in Figures 23 and 24, the changes of senility in man and rats * are similar to those after castration except that the non-uniformity of the structure described during the presenile period is not apparent. In senile mice non-uniformity of structure is found from the 480th to the 720th day, and in rats from the 650th to the 725th day. Figure 26 from the prostate of a 670 day old rat shows adjacent acini, some of which show advanced epithelial atrophy and others tall columnar cells. These morphological observations indicate that the changes of senile involution are due to the same causes as those of castration — namely decrease or absence of the hormones of the testis. This assumption is supported by the finding of a decrease of hormone secretion in old age (as measured by the urinary secretion) by Funk, Harrow and Lejwa [32] and the report of Womack and Koch [27] that the testis of the calf contains more hormones than that of adult or aged animals.

By the parenteral injection of the chloroform extractives of male urine † into castrated and senile animals, pictures similar to those of secondary hyperplasia may be produced.‡ In Figure 27 the combination of tall epithelium in a flattened acinus without papillae in an injected castrated rat should be compared with the picture of secondary hyperplasia in man in Figure 19. In some cases the variation in structure is striking with marked hyperplasia in one small focus, as shown in Figure 28, entirely similar to the lesion in man (Fig. 20).

From the combined morphological and physiological evidence the

* The prostates of 37 rats from 500 to 811 days of age have been studied. In addition, through the kindness of Dr. Jacob Furth, the prostates of 55 mice from 240 to 1140 days of age from the leukemia colony at Cornell University Medical College have been studied.

† The extraction of urine with chloroform has been carried out according to Harrow.

‡ A total of 44 white rats was injected with hormones at 10 day intervals, 5 with 50 units of "antuitrin S" after castration, 6 with the equivalent of 1000 cc. of male urine after castration, 12 senile with equal doses of "antuitrin S," and 21 senile with the same amount of male urine.

conclusion is justified that the senile involution of the mammalian prostate is due to a decrease and cessation of excretion of a hormone or hormones by the testis. Further, during the period of decrease, slight variations in the quantitative secretion combined with unequal reactivity of the tissue may result in a non-uniformity of structure.

Summary and Conclusions

1. At puberty there is a rapid maturation of the prostate, probably due to an internal secretion of the testis which is activated by the pituitary gland.

2. The mature postpuberal prostate is maintained as a uniform structure for about 25 years, except for occasional instances of pathological involution dependent on systemic disease.

3. During the 5th decade of life involution is initiated and continues as a progressive process into the 8th decade. All the evidence indicates that this involution is the result of a decrease and cessation of the same internal secretion which appeared at puberty.

4. There are occasional cases in which senile involution is delayed for 10 to 20 years beyond the average time. There is no adequate morphological explanation for these cases.

5. During the presenile period a non-uniformity of structure is characteristic and is probably the result of irregular stimulation combined with unequal reactivity of the tissues.

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DESCRIPTION OF PLATES

PLATE 114

FIG. 1. Early maturation of the epithelial cells from a child 10 years of age. The cytoplasm is more conspicuous, denser and more acidophilic than at an earlier age. × 225.

FIG. 2. The prostate from an individual 27 years of age showing a tall columnar epithelium with numerous papillae. × 85.

FIG. 3. Semidiagrammatic drawing of the prostate to show the division into lobes on the basis of the position of the urethra, deferential canal and prostatic ducts.

FIG. 4. A section of the prostate immediately cephalad to the opening of the ejaculatory ducts. Note the white lines, radiating laterally and posteriorly, which represent the ducts. There is cystic dilatation of the acini in the most peripheral parts.

FIG. 5. Low power photograph of an entire midsagittal section cephalad to the verumontanum. The acini are numerous and show many papillae. × 2.

FIG. 6. Type 1 cells (at top). The cytoplasm is slightly reticulated and each cell bulges into the lumen. The nucleus is round and moderately chromatic. There are occasional basal cells with elongated nuclei. × 710.

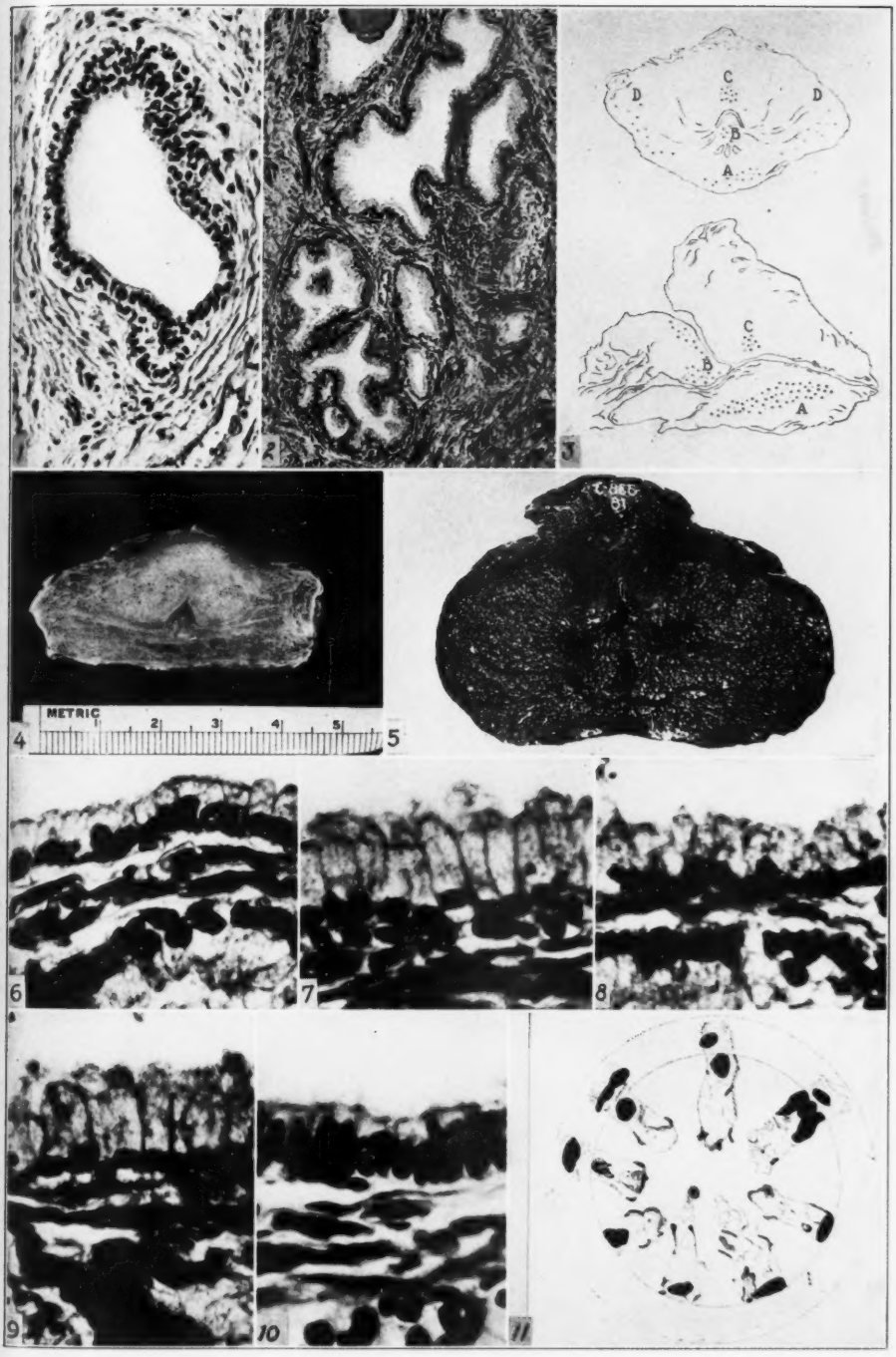
FIG. 7. Type 2 cells. The cells are tall with slight indistinctness of the luminal cell membrane. The nuclei are round and basal. The basal layer of epithelial cells is distinct with round nuclei. × 710.

FIG. 8. Type 3 cells. The cells are tall with a basal flattened highly chromatic nucleus. The basal cells are not evident. There is a beginning loss of the luminal cell membrane. × 710.

FIG. 9. Type 3 cells. The general features are the same as in Fig. 8 except that the luminal cell membrane is entirely lacking with discharge of a part of the cytoplasm into the lumen as secretion. × 710.

FIG. 10. Type 4 cells. The cells are moderately tall with elongated elliptical nuclei, the long axis of which is at right angles to the wall of the acinus. The cytoplasm is denser than in the other types and the luminal cell wall is smooth and distinct. × 710.

FIG. 11. Diagram of the cycle of secretion in the prostatic epithelium. Each cell in this diagram may be found in Figs. 6, 7, 8, and 9.



Moore Evolution and Involution of Prostate Gland

Plate 115

Fig. 12. Type 5 cells. The epithelial cells are cuboidal with definite cell walls and only very slight bulging of the cytoplasm into the lumen. × 820.

Fig. 13. Type 6 cells. The epithelial cells are low cuboidal and in thin sections there is overlapping of the nuclei. A basal cell is present only in rare areas. × 820.

Fig. 14. At the top there is complete atrophy of one lobule while below in other lobules there are acini with tall epithelium and numerous papillae.

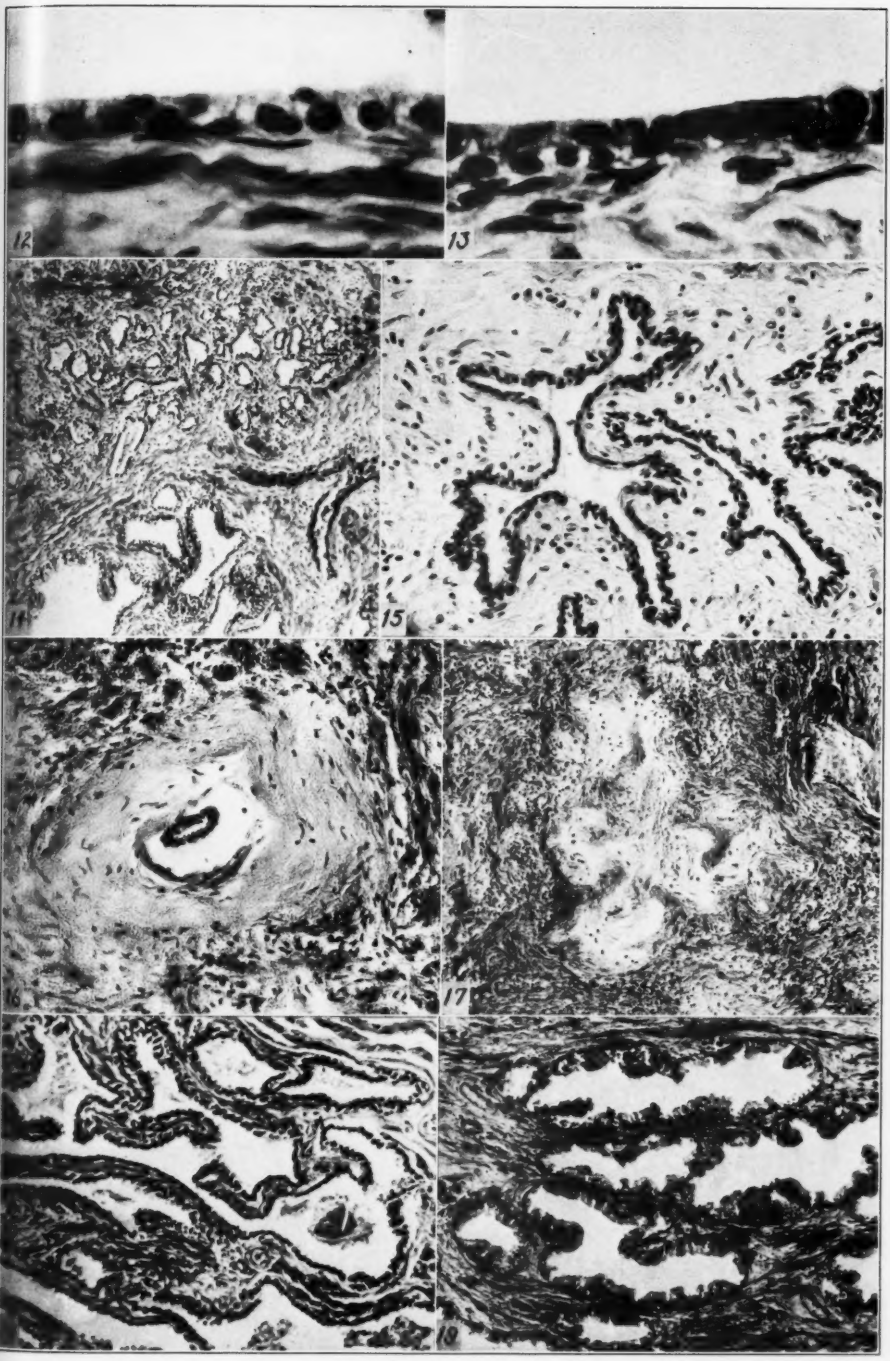
Fig. 15. Deformed acini in a senile prostate. The epithelium immediately over the connective tissue projections is flatter than in the depths of the clefts. The stroma adjacent is free of muscle fibers. × 215.

Fig. 16. A moderately advanced state of sclerotic atrophy. The epithelium is detached, probably due to fixation. There is a hyalinized collar about the former acinus. × 169.

Fig. 17. Complete sclerotic atrophy. The former acini are represented as irregular masses of loose tissue surrounded by a collar of hyalinized collagen. × 83.

Fig. 18. Involution of the prostate in a young man, 27 years old, with advanced pulmonary tuberculosis. The acini are flattened or irregular and the epithelium is low cuboidal. × 125.

Fig. 19. Secondary hyperplasia. The acini are separated and the epithelial cells are columnar. The papillae are atypical. There is moderate fibrosis of the stroma. × 125.



Moore

Evolution and Involution of Prostate Gland

PLATE 116

FIG. 20. Focal hyperplasia. In a local area the epithelium is tall with elliptical nuclei. There is no stromal reaction or nodule formation. × 30.

FIG. 21. A typical appearance of a posterior lobe of a prostate of an individual 70 years of age. × 85.

FIG. 22. The prostate in a rat 810 days of age. × 135.

FIG. 23. The prostate of a man 38 years of age who had been castrated 5 years previously. × 135.

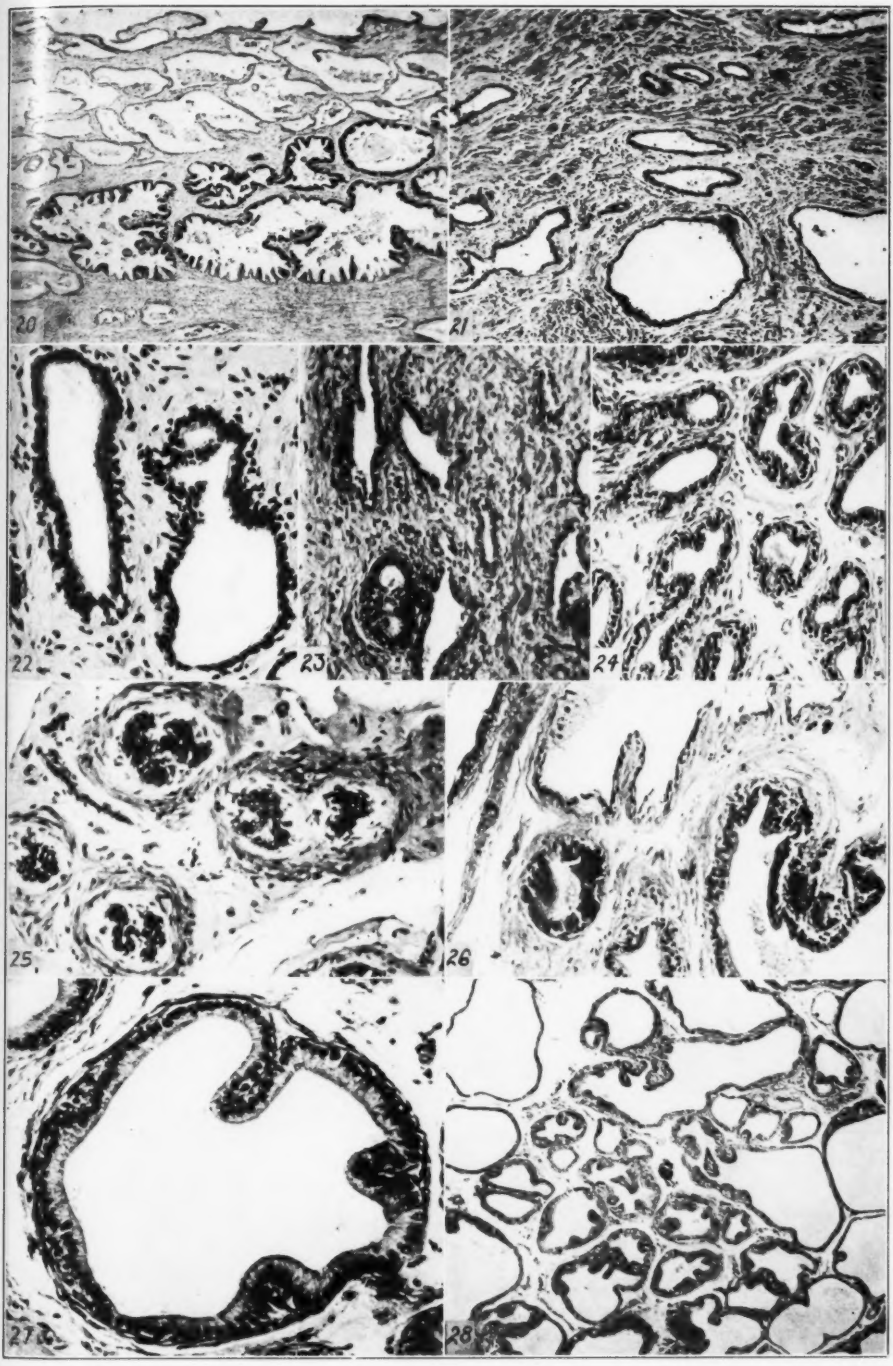
FIG. 24. The prostate of a rat which had been castrated 60 days before death. × 135.

FIG. 25. Hyalinized collars about acini in a rat 825 days of age. Compare with Figs. 16 and 17. × 135.

FIG. 26. The prostate from a rat 670 days of age. In the acinus above, the epithelium is extremely flattened while in three below it is tall columnar with evidence of secretion. Compare with Fig. 20. × 125.

FIG. 27. A castrated rat which had received for 10 days before death three bird units of the male sex hormone each day. The epithelium is tall but slightly irregular and the typical peripheral lighter areas are not present. × 200.

FIG. 28. A senile rat 800 days of age which had received ten injections of ten bird units of male sex hormone at intervals of 10 days. Note the irregularity in the structure of the acini and the numerous papillae in the central group. × 55.



Moore Evolution and Involution of Prostate Gland

CERTAIN CYTOPLASMIC INCLUSIONS OF LIVER CELLS*

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On routine examination of liver sections our interest has been aroused by the rather frequent occurrence of certain, well characterized cytoplasmic inclusions. Although easily seen, even with ordinary stains, they seem to have attracted little attention and to have escaped the recognition of experienced pathologists. They are not described in standard textbooks of pathology, histology or cytology. Mallory,[1] however, in his classical paper on necroses of the liver (1901) has accurately described and pictured these bodies (Plate XVI, Fig. 1, and Plate XVII, Fig. 1), and since we have found but one other reference to them his description may be cited in full.

"In a majority of the early cases the protoplasm of the liver cells contains numerous small and large vacuoles in which are single small hyaline globules; single, sometimes multiple, coarse, and fine threads; and occasional networks, all of which stain deeply with eosin and with Weigert's fibrin stain. With other stains they react exactly as fibrin does. The hyaline globules are not threads cut across; whether they really are fibrin is difficult to prove. They often seem to precede the formation of the threads and networks of fibrin."

It would seem that Mallory regarded these bodies as an accompaniment of early degeneration, ending in necrosis of the liver cells.

We have been able to locate but one other short reference to these structures. Taniguchi[2] (1931) describes in liver cells three types of cytoplasmic inclusions as follows:

Type 1: Spherical bodies, varying in size from a round mitochondrial granule to that of a nucleus, homogeneous and with sharp contours. They stain deeply with Heidenhain's iron hematoxylin after fixation in Regaud's fluid and can be distinguished, though less clearly, in hematoxylin-eosin preparations. They do not stain with Sudan III.

Type 2: More or less elongated oval bodies which are regarded as a modification of Type 1. They are infrequently found.

* Received for publication March 25, 1936.

Type 3: Comparatively narrow, long and short, thread-like, slightly bent, sometimes spindle shaped bodies. They are derived from swollen, thread-like mitochondria.

The material for Taniguchi's study was obtained from surgical biopsies. The bodies described as Type 1 were found in 27 of 94 cases. The photographic illustrations accompanying the paper show, though not very clearly, spherules and filaments.

We have been interested in making a somewhat more extended study of these bodies.

Description of the Inclusions

(*A*) *Spherical Bodies:* Our observations in the main confirm the description given by Mallory. The spherules vary in size from that of a small micrococcus up to 4 or 5 μ in diameter, the majority ranging from 1 to 3 μ. They lie in a large vacuole which, with the usual staining methods, is optically empty or contains only a little shredded material. When the vacuole is contiguous to a nucleus the nuclear membrane may be indented by it. There may be but a single spherule or the entire cytoplasm may be riddled with vacuoles, each containing a single body, so that the cell has a cribriform or porous structure. Adjacent vacuoles may coalesce into large spaces containing several spherical inclusions. When they are on the surface they may rupture, discharging the inclusion body into the space between liver cell and sinus wall.

Staining Reactions

With *hematoxylin-eosin* staining, the spherical bodies are acidophilic, but the color is unlike that of the cytoplasm or of the erythrocytes, having a brownish yellow cast. They are sharply contoured, moderately refractive, and entirely homogeneous without any suggestion of internal structure.

With the *Gram* stain the methyl violet or gentian violet is retained, if decolorization is not pushed too far. The degree of Gram positiveness is about the same as that of the nucleolus.

Mallory's phosphotungstic acid hematoxylin stains the spherules very selectively a bluish black.

After formalin fixation, staining with *phosphotungstic* and *hematoxylin* demonstrates that the spherule is surrounded by a homo-

geneous capsular material which stains a dull brick red and completely fills the vacuole (Fig. 3).

Heidenhain's iron hematoxylin also brings the bodies out sharply but they are resistant to decolorization with ferric chloride.

With *azocarmine, anilin blue* and *orange G*, the bodies take a dull orange color similar to that of the erythrocytes.

With *methyl green-pyronin*, both spherules and rods take only a faint yellowish stain. With *mucicarmine* the globules do not take the mucin stain.

Masson's trichrome stain shows the spherules to be red, in contrast to the dark violet gray of the cytoplasm. The nucleoli are bluish black. Contents of the vesicle are pale blue. There is no unstained vacuole. The bluish material is sometimes homogeneous, sometimes finely granular (Fig. 4). The rods stain orange-red and lie in a clear vacuole without the bluish staining matrix.

Mallory's anilin blue collagen stain shows about the same picture as that of Masson's, except that the spherules are orange-red and the bluish matrix is less sharply brought out.

With *Mann's* stain the spherules are dull red to bluish purple, depending on the degree of differentiation. Filaments stain about the same. Contents of vacuoles remain unstained (Fig. 1).

With *Giemsa*, they are stained a rose pink.

The most selective stain that we have found is *Laidlaw's* method for demonstrating virus inclusions.* By careful differentiation with orange G alcohol the cytoplasm of the liver cells may be decolorized to a grayish blue: the spherical inclusions retain the fuchsin and stain an intense crimson. The red blood cells are a tawny orange-red (Fig. 2). While Laidlaw's method calls for fixation in acetic

* LAIDLAW'S METHOD FOR STAINING INCLUSION BODIES

1. Fixation in { saturated aqueous corrosive sublimate ..... 100 cc.
   { glacial acetic acid ..... 5 cc.
2. Embed in paraffin and cut at 3 μ.
3. Deparaffinize in xylol, absolute and 95% alcohol and rinse in water.
4. Stain in Weigert's iron hematoxylin (2%) 5 minutes.
5. Differentiate in 0.5% acid alcohol.
6. Rinse in tap water followed by distilled.
7. Stain in 1% aqueous acid fuchsin 5–15 minutes.
8. Rinse in distilled water.
9. Mordant in 1% phosphomolybdic acid for 30 seconds.
10. Rinse in distilled water.
11. Differentiate in 0.25% orange G in 70% alcohol.
12. Dehydrate, clear and mount in balsam.

acid bichloride, Zenker's fixation without acetic acid gives equally brilliant pictures.

The bodies are not stained by *scharlach R.*

(*B*) *Rod Shaped and Filamentous Structures:* These occur almost always in association with the spherical bodies, but far less frequently (only in about one-fifth of the cases). They also lie in clear vacuoles and assume a variety of forms — simple bacillus-like rods, beaded or fuzzy straight rods, or long curving fibrils, sometimes looped upon themselves. Small crossing or interlacing filaments may be seen within the same vacuole.

Not infrequently the fibrils seem to be formed directly on or from the spherical body; the spherules may lie in the middle or at the end of the rod. But in many of the vacuoles containing rods or filaments, no spherical body can be discerned.

The staining reaction of these filaments and rods, as Mallory pointed out, is the same as that of fibrin. They retain the gentian violet in the Gram-Weigert stain, and stain bluish black with phosphotungstic acid hematoxylin. They are also brought out by the Heidenhain iron hematoxylin method and by the Laidlaw stain.

General Incidence

In all, 562 sections of liver from human cases have been studied. The spherical inclusions were found in 175 cases, or 31.1 per cent. Many blocks were recut and stained with phosphotungstic acid hematoxylin, and in these preparations the bodies are more easily recognized. Their incidence in the hematoxylin-eosin sections was therefore somewhat lower (23.7 per cent) than in the 124 sections stained with phosphotungstic acid hematoxylin, which gave 43.6 per cent of positives.

Included in the 562 cases are 391 adults, 124 children, and 47 newborn infants and stillbirths. The percentage incidence in the different age groups is shown in Table I.

TABLE I

Age	2 wks.	2 wks.–1 yr.	1–5 yrs.	6–10 yrs.	11–20 yrs.	21–30 yrs.	31–40 yrs.	41–50 yrs.	51–60 yrs.	61–70 yrs.	71–80 yrs.	80+ yrs.
No.	47	46	30	18	30	42	66	79	104	65	32	3
No.+	14	6	7	6	9	14	27	23	40	17	10	2
%+	29.7	13.0	23.3	33.3	30.0	33.3	41.0	29.1	38.4	26.1	31.2	66.6

There is, as the analysis shows, no significant difference in the various age groups. The spherules were found once in a stillborn full term infant, and in several prematures.

Occurrence in the Human Fetus

Through the courtesy of Dr. Coler of the Sloane Hospital, we have been able to obtain material from human fetuses from 5 months to term. The sections stained with phosphotungstic and hematoxylin showed an astonishingly high incidence of inclusions.

Age of Fetus	*No. Examined*	*Positive*	*Negative or Doubtful*
5 mos.	7	7	..
6 mos.	7	4	3
7 mos.	7	6	1
8 mos.	6	5	1
Full term	7	4	3
	34	26	8

The failure to identify the bodies in a few of the cases may have been due to poor preservation of the tissue. In 8 of the 34 cases, that is in about the same proportion as in the postnatal livers, the spherules were associated with rods and filaments.

Relation to Possible Postmortem Changes

The bodies seem to be relatively little affected by autolytic changes, and they do not become more numerous after death. They have been found as early as 45 minutes portmortem and as late as 53 hours. The incidence of positive findings at various periods after death is shown in Table II.

TABLE II

Hours postmortem	2	2–6	6–12	12–24	More than 24
Total autopsies	26	165	130	146	43
No. showing globules	6	50	45	39	16
No. showing rods	1	12	13	9	4
% globules +	23.1	30.3	34.6	26.7	37.2
% rods +	3.8	7.2	10	6.2	9.3

Although there are slight variations in the different groups, there is no trend toward a definite increase or decrease in the incidence, as the period elapsing after death becomes greater.

Relation to Other Diseases

We have attempted to correlate the occurrence of the spherules with various general diseases such as arteriosclerosis, nephritis, and acute and chronic infections. As might have been anticipated from the rather uniform age distribution and the presence of the bodies in the fetal liver, no such correlation seems to exist. The bodies have been present in all sorts of pathological conditions, and a detailed analysis of our material from this point of view would be futile. The only reservation to the above statement is in the case of malignant disease. Among the 562 cases examined, 111 had malignant tumors, and of these 24 showed liver inclusions — an incidence of 21.6 per cent as against an incidence of 33 per cent in the 451 non-tumor controls. The significance of this lowered occurrence is not apparent, but the difference would appear to be too great to ascribe to chance.

Not only is there no clear-cut correlation with general diseases, but we have been able to find no constant association with any particular type of liver lesion. The inclusions are found frequently in otherwise normal appearing cells. When they are very numerous the cytoplasm of the cells containing them has a spongy or cribriform appearance. They are always more abundant in the central portion of the lobule and often seem to be especially numerous when the liver cells are atrophic, or in the presence of central congestion. But they are quite definitely not associated with necrosis of the cells and we cannot interpret their presence as an indication of early degeneration or cell death. On the other hand, they may often be recognized in cells that have recently undergone necrotic changes. We have not found them in the neoplastic cells of primary liver cell carcinoma.

Occurrence in Laboratory Animals

In monkeys the spherical inclusions have been found in 1 of 5 livers examined. In guinea pigs they have been seen in 8 out of 44 livers — slightly less frequently than in human livers. In 7 ferret livers they were present in every instance. Rods and filaments, however, have not been observed.

On the other hand, we have searched in vain for these bodies in the livers of rabbits, dogs, cats, pigs, rats, mice, chickens or ducks.

Discussion

It has not been possible to arrive at any final conclusion as to the nature of the spherical inclusions. Several possible interpretations come to mind and may be discussed briefly:

1. They are some unknown structural component of the cell, like the Golgi net, centrosome, or mitochondria.
2. They are secretory products.
3. They are degenerative products, akin to the colloid droplets in degenerative renal epithelial cells.
4. They are cytoplasmic "virus inclusions."

It is difficult to believe that these structures represent some unknown organs of the cell. Were such the case, one would expect to find them in every cell, certainly in every liver and in every species. Nor is there any reason to believe them to be derived from known cytoplasmic constituents. They stain quite differently from mitochondria, persist long after the mitochondria have been destroyed by autolysis, and are not affected by acid. There is nothing to suggest that they are related to the Golgi net or centrosome, and they are most certainly not due to extrusion of nuclear chromatin.

We can find no particular evidence for or against the possibility that they represent a secretory product of the liver cells. The fact that with Gram and phosphotungstic acid hematoxylin the staining is like that of fibrin, brings up the thought that they may be associated with the production of fibrinogen. But the staining for fibrin is far from being specific and it would require much more evidence to justify such an assumption.

Against the secretory nature of the product is the observation that only a single body may be present in the cell. We can recall no instance in which secretory granules were not multiple. Perhaps it may be taken as a point also against the secretory nature of these bodies that they are not found in all human livers, as one might expect if they represent a normal activity of the liver cells, and that they are not demonstrable in the livers of dogs, rats, rabbits, cats, chickens or ducks.

Against the view that the spherules represent a degenerative alteration in the cytoplasm is the fact that they are often found in cells that show no other evidence of injury. It is true that they are most numerous in the central portions of the liver lobules, where atrophy

or necrosis accompanying central congestion is so frequently seen. Doubtless also, their presence in great number, with the resulting spongy rarefaction of the cytoplasm, may lead to the distintegration of some of the cells. On the other hand, the bodies may be found in cells adjacent to the portal spaces, and in this situation the liver cells often show no evidence whatever of damage.

The last possibility which comes to mind is that the bodies are of the nature of virus inclusions. This assumption would lead of necessity to the view that a large proportion of human livers harbor a non-pathogenic virus or viruses, and that similar viruses occur in the livers of monkeys, guinea pigs and ferrets. The fact that we have found the inclusions in the liver cells of the fetus would also logically involve the assumption that the hypothetical virus is transmitted to the embryo.

The only evidence in favor of these bodies being virus inclusions is (1) their morphology, which is not unlike that of certain cytoplasmic inclusions, such as the Guarnieri bodies (*cf.* Cowdry, E. V., *J. Exper. Med.*, 1922, **36**, 666, Fig. 33); and (2) the staining reactions. We have had opportunity to compare the liver cell inclusions with cytoplasmic virus inclusions in the various tissues of ferrets experimentally infected with a virus now being studied by Dochez, Slanetz and Smetana.* The staining of the liver cell inclusions is practically identical with that of the virus inclusions, but the latter are not surrounded by a stainable halo.

It would be of interest to obtain experimental support for the virus theory, as the demonstration of such a frequently occurring, latent, non-pathogenic virus would be of great theoretical importance. Unfortunately we have thought of no obvious way of attacking the problem experimentally at the present time.

As regards the rod-like and filamentous inclusions, we agree with Mallory that they are probably fibrinous in nature. They occur in about one-fifth of the livers containing the spherical inclusions, but only in association with them. They may occupy the same vacuole as the spherical bodies, and indeed one frequently gets the impression that filaments have formed on the spherical bodies as a nucleus. But often the rods and filaments occur in vacuoles that contain no spherical body. The only plausible explanation for their occurrence is that coagulable fluid from the plasma has seeped into the

* We are indebted to Dr. Smetana for placing this ferret material at our disposal.

vacuoles from the adjoining sinusoids and has crystallized out within the vacuoles. Why this does not occur in every case is not easy to understand.

Summary and Conclusions

A study has been made of certain spherical, rod-like and filamentous cytoplasmic inclusions that occur frequently in the liver cells of man, monkeys, ferrets and guinea pigs. They have not been seen in the livers of other laboratory animals. While no decision has been reached as to their nature and significance, various possible interpretations are discussed.

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DESCRIPTION OF PLATES

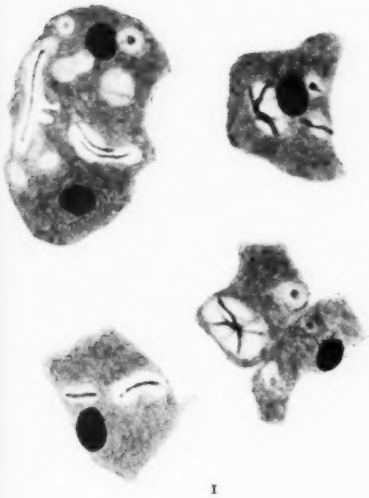
PLATE 117

FIG. 1. Liver cells containing spherical and filamentous cytoplasmic inclusions. Mann's stain.

FIG. 2. Spherical inclusions. Laidlaw's stain.

FIG. 3. Spherical inclusions surrounded by a homogeneous, slightly reddish substance. Formalin fixation, phosphotungstic acid hematoxylin stain.

FIG. 4. Spherical inclusions surrounded by bluish staining material. Masson's trichrome stain.



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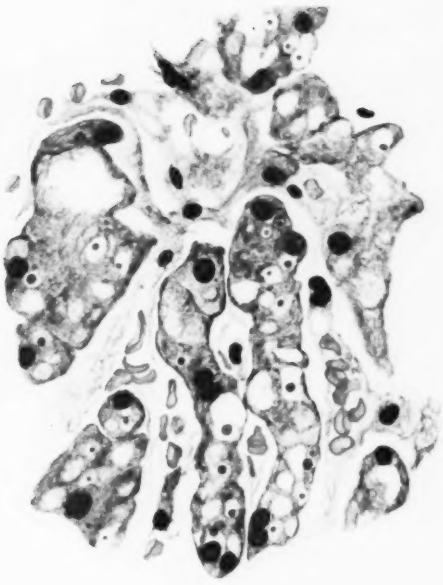

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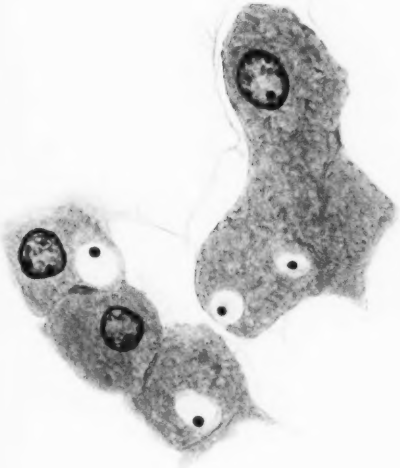

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4

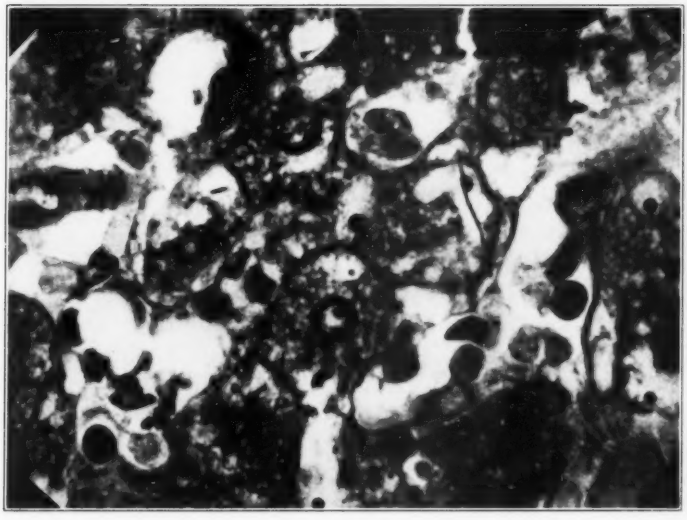
Pappenheimer and Hawthorne Cytoplasmic Inclusions of Liver Cells

PLATE 118

FIG. 5. Spherical inclusions in liver cells. Note absence of degenerative changes in nucleus. Phosphotungstic acid hematoxylin stain. × 1050.

FIG. 6. Spherule with long filament attached is seen to right of center. Smaller rod-like structures and spherules are seen in other cells. Phosphotungstic acid hematoxylin stain. × 1050.

5



6

THE RELATION OF AGE AND HYPERTENSION TO THE STRUCTURE OF THE SMALL ARTERIES AND ARTERIOLES IN SKELETAL MUSCLE *

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This study was made to determine whether or not age or chronic hypertension produces any characteristic changes in the structure of the small arteries and arterioles in skeletal muscle.

Many observations of the arterial changes in hypertension have been made. In 1868 Johnson examined the small arteries in 10 cases of chronic Bright's disease. Since he did not consider that the vessels of the kidneys alone could increase peripheral resistance, he also studied those of the pia mater, brain, intestine, subcutaneous tissue and muscle, and stated that in each the media of the arteries was hypertrophied. Four years later Gull and Sutton described what they called "arterio-capillary fibrosis" in Bright's disease. This was said to consist of a hyalin-fibroid substance which was formed external to the muscular layer. It was thought to be a morbid change. These authors stated that the arterioles were often very much thickened and tortuous. They drew an outline of the vessel with a camera lucida and measured the ratio of the thickness of the wall to the diameter of the lumen. In cases of kidney disease the usual finding was a lumen equal to twice the width of the wall. A normal relationship was not established. The muscle was said not to be increased. They thought that the lesions in the arterioles of the kidneys were identical with those found in the arterioles of other organs. This alteration of the arterioles described by Gull and Sutton does not correspond with anything seen when modern technical methods are used.

In 1877 Ewald confirmed Johnson's findings. He examined only the vessels of the pia mater in the region of the pons and also determined the wall to lumen ratio which in normal individuals was 0.1 to 0.3: 1.0. In cases of contracted kidneys with hypertrophy of the left ventricle the ratio rose from 0.3 to 1.2: 1.0. The increase in

* Received for publication February 6, 1936.

the thickness of the wall was due to a simple increase in the number of muscle fibers.

Jores, in 1904, examined the small arteries in cases of contracted kidneys with cardiac hypertrophy. He was unable to find any change whatever in the vessels of skeletal muscle except in 1 case of adhesive pericarditis and aortic insufficiency in which the circular muscle of the media appeared to be thickened.

Evans, in 1920, described diffuse hyperplastic sclerosis of the renal arteries in chronic nephritis. Under this term he included the fatty or hyaline swelling of the intima, hypertrophy of medial muscle and perivascular fibrosis. He also examined the arterioles of various other organs including the skeletal muscle in 31 cases. In the arterioles of muscle he found changes in only one patient, an irregular intimal thickening which he interpreted as being a syphilitic lesion.

In a study of 72 cases of hypertension Fishberg found lesions in the small arteries in 100 per cent of the kidneys. Arteriolosclerosis was present in the spleen in most of the cases. It was found in a smaller percentage of the cases in the pancreas, liver, adrenals, brain and gastro-intestinal tract. Arteriolosclerosis was never observed in the skeletal muscle in this series.

In 1927 Fishberg described the necrotizing arteritis, endarteritis obliterans, and arteriolosclerosis seen in the kidneys in acute and chronic glomerulonephritis. He was unable to find changes in the muscle of the media in early nephritis except in the necrotizing endarteritis seen occasionally in acute glomerulonephritis. In chronic nephritis he found a thickening of the medial muscle layer which was often most marked in the arcuate and interlobular arteries. He thought that this was due to hypertrophy and hyperplasia of the muscle cells without fibrosis. He did not state what staining methods were used. In renal arteriolosclerosis and chronic glomerulonephritis of long standing the muscle of the media was usually atrophied.

Branch and Linder in 1926 studied the arterioles of the skeletal muscle in 6 cases of chronic nephritis and were unable to demonstrate any changes. Theodore Fahr has stated that hypertension is secondary to renal arteriolosclerosis. He found no changes in the arterial systems of the skeletal muscle or skin.

In 1928 Keith, Wagener, and Kernohan described what they called "malignant" hypertension, and noted widespread arterial lesions in these cases. In the following year Kernohan, Anderson

and Keith studied the arterioles of pectoral muscle in cases of hypertension, and in 1931 Keith, Barker and Kernohan reported studies of the arterioles from 143 patients with hypertensive disease. Skeletal muscle was chosen for study because it comprises 30 to 40 per cent of body weight, and pectoral muscle was chosen because it is easily accessible for biopsy. It was thought that a generalized vascular change would of necessity affect these vessels. Arteries having an outside diameter of from 25 to 100 microns were measured and the ratio of the thickness of the vessel wall to the diameter of the lumen was determined. The normal ratio was found to be 1.0:2.0 with a variation of 1.0: 1.7 to 1.0: 2.7. They considered the most striking changes in hypertension to be a thickening of the media which was interpreted as due to an increase in the number of muscle fibers. They found no increase in the connective tissue of the media and thought that the medial change was due entirely to a proliferation of muscle. The internal elastic lamina was also hypertrophied. In some cases it was split but usually not to a pronounced degree. They also found proliferation of the lining endothelium of the intima which was sometimes accompanied by proliferation of the subendothelial connective tissue. They saw no degenerative changes which they could attribute to senile retrogression. They classified their cases as "benign," "severe benign," and "malignant" hypertension. In benign hypertension the wall to lumen ratios were 1.0: 1.1 to 1.0: 1.8, with an average of 1.0: 1.4. Cases of severe benign hypertension and malignant hypertension showed practically the same ratios, an average of 1.0: 1.1 with variations from 1.0: 0.9 to 1.0: 1.7. These authors use the term "arteriolosclerosis" to describe these medial changes though most writers restrict the use of the term to those arterioles in which intimal changes predominate.

In 1932 Horine, Weiss and Beard reported a case of an 88 year old negro who was first seen in congestive heart failure. The blood pressure was then 130/80. Biopsy of pectoral muscle 9 days before death showed a wall to lumen ratio of 1.0: 0.96. At postmortem the vessels of the same muscle had a ratio of 1.0: 0.93. There was also reduction of the relative size of the lumen in the small arteries of the heart, liver, spleen, pancreas and kidney.

In a study of the arterioles of pectoral muscle of 153 of 375 patients having essential hypertension, Murphy, Grill, Pessin and Moxon found moderate to advanced hypertrophy of the media in the art-

erioles of 57 of 124 of the persons having high blood pressure and arteriosclerotic disease. Hypertrophy of the medial muscle was observed in all of the 29 patients who had malignant hypertension. The degree of hypertrophy varied from "slight" to almost complete closure.

Scott, Seecof and Hill graded arteries of skeletal muscle in patients suffering from arterial hypertension according to thickening of the vessel wall, hyalinization of the vessel wall, other changes such as fatty, fibrous and necrotizing lesions, and size of lumen. They were able to demonstrate arteriolar lesions in skeletal muscle in a very high percentage of individuals dying under 46 years of age of diffuse vascular disease with hypertension. In 68 per cent of 386 autopsied cases there was a relation between the severity of the arteriolar lesions in the kidney and those of skeletal muscle. Necrotizing arteriolar lesions were not noted in skeletal muscle. They found that the lesions in biopsy specimens agreed closely with those found in a postmortem specimen of the same muscle.

Pilcher and Schwab, following the work of Keith and his coworkers, made measurements of arterioles of the kidneys, liver, pancreas, spleen and myocardium in 15 cases of hypertension. There was uniformly a reduction in the wall-lumen ratio except in the myocardium where the ratio was lowered in 4 cases. These authors, however, believe that the increased peripheral resistance cannot be regarded as resulting from thickening of the walls of the arterioles. They cite the absence of true generalized arteriolosclerosis, the fact that clinically hypertension precedes the arteriolar changes, the acuteness of the condition in acute nephritis and urinary obstruction, and the variability of arterial pressure as proof that hypertension must result from a functional vasoconstriction of the arterioles, and that the arteriolar lesions are the result of the elevation of blood pressure.

The part that arterioles of the somatic area may play in the regulation of blood pressure is by no means established. Dawson, von Bonsdorff and others have shown that the blood pressure is fairly well sustained until the small muscular arteries are reached. Landis has determined the capillary pressures directly, and Voldeng, by making indirect measurements of the capillary pressures, showed that the values in normal subjects and in persons suffering from hypertension are very nearly the same. It may be seen therefore

that the pressure gradient is greatest in the small arteries and arterioles. When Jansen, Tams and Achelis put tourniquets on three extremities and measured the blood pressure on the free arm the blood pressure rose only 6 per cent or not at all, while the same procedure in hypertensives caused a sustained rise in blood pressure of 15–20 per cent. This phenomenon was interpreted by them to indicate that the splanchnic vessels in patients with hypertension lose their ability to distend and thus compensate for constriction of peripheral vessels. It is more probable that the greater rise was due to the more exaggerated responses to painful stimuli made by persons with hypertension. Steele and Kirk point out the fact that observations on patients with Buerger's disease confirm the experimental findings that no relation exists between the obstruction of the arteries of extremities and the level of systemic blood pressure.

The rise in arterial pressure following the injection of adrenalin is caused by constriction of the arterioles of the skin and abdominal viscera. At the same time there is dilatation of the arterioles of skeletal muscle and probably also of the heart muscle. Because of the relatively numerous vasomotor fibers to the arterioles of the skin and splanchnic area, it is probable that the contraction of the arterioles of these regions may account for some other elevations in blood pressure.

Jansen and his co-workers found also that cold colonic irrigations were followed by a rise in blood pressure of 22.5 per cent while a cold bath caused a rise in blood pressure of only 8 per cent. Ligature of the renal arteries of animals causes no rise in blood pressure, but ligation of the superior mesenteric artery causes a marked rise in blood pressure. The action of the arterioles in the splanchnic area has a profound effect on the level of arterial pressure.

In 1924 Brogsitter investigated the mesenteric arteries in normal individuals and in 33 cases of persistent hypertension. In some of the larger arteries he found nodular, cellular and fibrous intimal proliferations and homogeneous areas in the media. In others there was a localized thickening of the adventitia with reduction of muscle in the media and a cellular intimal proliferation. He interpreted these lesions as the healing of an inflammatory process with scar formation. Severely altered vessels were found in only a few persons. Besides the vessels with changes there were always others of the same size in the same mesentery which were entirely normal.

There was no constant relation between the arterial changes and the presence of hypertension.

Moldenhauer determined the blood pressure in patients having malaria and found that during the cutaneous vasoconstriction of each chill the arterial pressure rose only 5 to 20 mm. Hg. Steele and Kirk found that the attacks of paleness and numbness of the skin in hypertensive individuals resulting from constriction of arterioles are not accompanied by rise in blood pressure. They measured skin temperatures on nine hypertensives and found that the form of the curve of surface temperatures in a 24 hour period did not differ from the curve obtained from normal individuals and that the level of the two groups was approximately the same. When the arterial pressure is high, some increase in tone must develop in order to maintain a normal flow of blood through the skin. The latter is known to be true by the fact that the skin temperature is normal. If there were no increase in tone, excessive loss of heat would result in the presence of high arterial pressure. These workers regard the increase in tone of skin vessels as dependent on, rather than the cause of, general arterial hypertension. Watanabe was able to demonstrate sclerosis of the small arteries of the skin in only 21 of 116 persons when 4 specimens of skin from each subject were studied. In only 2 of the cases were there findings in more than 1 specimen and in only two sites in those. Of the 21 persons, only 2 had myocardial hypertrophy and these had emphysema. From these findings it may be seen that constriction of the cutaneous vessels alone has little or no effect on the arterial pressure. Their structure is not altered in hypertensive states.

The term "arteriole" does not have an exact meaning in the literature. Evans defined an arteriole as an artery with a media of two or three muscle cells in thickness. The afferent glomerular arterioles have an outside diameter of 25 to 45 microns. Maximow and Bloom described an arteriole as an artery having a simple endothelial lining lying next to the internal elastic lamina, and a media that is composed only of muscle. The adventitia equals the media in thickness. They state that the internal elastic lamina becomes thinner as the vessel becomes smaller and becomes invisible when the vessel has a diameter of 62 microns. When the vessel becomes 27 microns in thickness it loses its muscular coat. The adventitia loses its elastic fibers and becomes transformed into a thin collage-

nous membrane with scattered fibroblasts and gradually passes over into the perivascular cells of the capillaries.

Personal Observations

For this study 137 specimens of pectoral muscle taken from routine autopsy material were examined. Arteries having an outside diameter of 15 to about 150 microns were studied. Some of the vessels with a diameter above 100 microns would be called small arteries by many authors. The same criteria that Bell and Clawson used in their study of hypertension were employed to determine the presence of hypertension. Persons with heart weights of over 450 gm. in females and over 500 gm. in males in the absence of valvular disease, glomerulonephritis, pericarditis or hyperthyroidism were taken as cases of hypertension. All persons in whom the systolic blood pressure was known to have been 150 mm. Hg. or more were included in the hypertensive group regardless of the weight of the heart. There were 32 cases in the hypertensive group. The heart weight was above the limits of normal weight in 20 cases. There were no instances of normal blood pressures when the heart weight was above normal. In 14 cases the blood pressures were not recorded, and in 12 cases the blood pressures were above 150 mm. systolic, although the heart weights were less than 450 gm. in the female and 500 gm. in the male. There was a total of 40 females and 97 males in the entire series. Muscle was examined from one stillborn infant. Pieces of pectoral muscle were fixed in 10 per cent formalin, embedded in paraffin, and stained with Heidenhain's modification of Mallory's aniline blue collagen stain. This stain distinguishes muscle cells sharply from all types of connective tissue. Muscle cells are colored red; collagenous, elastic and reticular fibers are blue. Some of the sections were also stained with hematoxylin-eosin, Weigert's elastic tissue stain and the Van Gieson stain.

There is no diameter at which the structure of the vessel wall suddenly changes and permits one to distinguish an arteriole from a small artery. Arteries with an outside diameter of less than 150 microns have a simple endothelial lining which lies directly upon the internal elastic lamina. No nuclei or fibers were seen between the endothelium and the internal elastic lamina in any instance. The internal elastic membrane was not seen to be split in any of

the vessels of skeletal muscle up to 100 microns in diameter. Hyalinization of the intima was never observed.

The Mallory-Heidenhain (azocarmine) stain is used to great advantage in the study of the media, for it stains not only the coarse collagenous fibers, but also the reticulum. (Mallory's aniline blue stain does not stain reticulum fibers.) It may be seen here, as Mallory and Parker showed by the use of silver impregnation methods in leiomyoblastomas, that each muscle cell is separated from the surrounding ones by a layer of reticulum. This may be seen in Figure 1. The most marked variations encountered in the small arteries and arterioles of skeletal muscle were in the relative amounts of connective tissue fibers in the media. Mallory and Parker state that this reticulum is collagen which is divided into fine strands and thin layers. When in this finely divided state reticulum is stained by silver impregnation methods; when it occurs in coarse bundles it stains like collagen. They found that with necrosis of the muscle cells of smooth muscle tumors the reticular fibers become compacted and give the staining reactions of collagen.

In my material no areas of hyalin were observed in the media of any of the small arteries or arterioles. All of the fibers run in a circular or longitudinal direction. Radial T or Y shaped fibers like those Dietrich described in the larger arteries are not found in the arterioles. There are few elastic fibers in the media. Fibroblasts are not seen in connection with the reticulum. In cases of marked fibrosis of the media the muscle cells disappear and the wall is almost completely composed of blue staining material when stained with the Mallory-Heidenhain stain.

The medial change described above is known in the literature as medial fibrosis. In 1913 Ssobolew described fibrosis of the media in the aorta and iliac artery which he called "cirrhosis of the vessel wall." Staemmler studied the fibrosis of the renal and splenic arteries in relation to the age of the individual. He found an increase in the connective tissue of the media in the third decade. With increasing age the amount of connective tissue in the media becomes more marked. Dietrich found an increased amount of collagen with increased age. Troitzkaja-Andreewa found that the collagenous fibers not only become larger but more numerous. He noted that the precollagenous fibers were transformed into collagen and in advanced age the coarse bundles of collagen lose their sharp

TABLE I

Cases Without Hypertension. The Degree of Fibrosis of the Media is Estimated

Case No	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	yrs.			*gm.*		
1	0	F	?	?	10	Asphyxia
2	14	F	?	225	30	German measles
3	18	F	140/100	275	30	Uremia, chronic nephritis
4	19	M	?	200	30	Miliary tuberculosis
5	21	M	?	375	20	Trauma
6	23	F	?	300	20	Septic abortion
7	24	M	?	365	40	Fracture of neck
8	27	F	112/74	280	30	Leukemia
9	28	M	?	305	20	Trauma
10	29	F	110/80	250	60	Pneumonia
11	29	M	?	355	30	Trauma
12	29	M	110/60	250	20	Miliary tuberculosis
13	29	F	114/70 170/125	190	40	Toxemia of pregnancy
14	30	M	?	300	60	Trauma
15	31	F	?	350	50	Hemorrhage from peptic ulcer
16	32	F	?	175	20	Lobar pneumonia
17	35	F	?	250	50	Pneumonia
18	36	M	?	425	30	Portal cirrhosis
19	36	M	?	280	30	Lipoid nephrosis
20	37	M	?	260	30	Undetermined
21	38	M	?	300	50	Meningitis
22	38	F	?	650	30	Rheumatic heart disease
23	39	M	130/70	320	50	Hodgkin's disease
24	39	M	122/65	275	50	Lobar pneumonia
25	40	M	?	250	20	Partial intestinal obstruction
26	40	F	?	300	50	Peritonitis
27	41	M	?	400	50	Coronary arteriosclerosis, old valve defect
28	41	M	?	295	30	Pneumonia
29	42	M	?	425	30	Fracture of skull
30	43	M	?	375	30	Phenobarbital poisoning
31	43	M	?	400	30	Pneumonia
32	44	F	?	260	50	Mesenteric thrombosis
33	45	F	?	250	40	Purulent bronchitis
34	45	M	?	275	20	Septicemia
35	45	M	?	310	40	Trauma
36	46	M	?	225	30	Postoperative hemorrhage
37	46	M	132/80	375	30	Pulmonary embolism
38	47	M	?	300	40	Obstructive jaundice
39	47	M	?	425	30	Trauma
40	48	M	?	380	30	Fracture of neck
41	48	F	?	430	30	Pulmonary tuberculosis
42	50	M	?	400	40	Coronary occlusion
43	49	M	?	360	30	Subdural hematoma
44	50	M	80/50	300	80	Pulmonary tuberculosis

TABLE I (*continued*)

Case No.	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	yrs.			*gm.*		
45	50	M	?	460	30	Coronary sclerosis
46	51	M	?	300	30	Appendiceal abscess
47	52	M	?	400	50	Trauma
48	52	M	?	275	30	Pancreatitis
49	52	M	?	385	30	Carbon monoxide poisoning
50	53	M	?	450	30	Aortic aneurysm
51	54	M	138/90	375	50	Carcinoma of rectum
52	54	M	?	450	30	Coronary occlusion
53	54	M	?	300	30	Trauma
54	55	M	?	400	20	Coronary occlusion
55	55	M	?	385	70	Coronary thrombosis
56	55	M	?	400	40	Coronary thrombosis
57	55	M	?	410	40	Perforated gastric ulcer
58	55	M	135/60	350	50	Carcinoma of pleura
59	55	M	?	365	50	Coronary sclerosis
60	55	M	125/85	275	20	Lobar pneumonia
61	56	M	?	400	20	Meningitis
62	57	M	?	250	30	Bronchopneumonia
63	57	M	?	338	50	Undetermined
64	59	M	129/88	475	30	Coronary sclerosis
65	60	F	?	400	50	Portal cirrhosis
66	60	M	?	375	30	Acute leukemia
67	60	M	?	350	30	Syphilitic encephalitis?
68	61	F	?	400	50	Carcinoma of stomach
69	61	M	?	360	40	Trauma
70	62	M	?	300	30	Myelitis
71	62	F	?	300	20	Peritonitis
72	62	M	?	350	80	Hemorrhage
73	63	M	116/100	440	50	Trichlorethylene poisoning
74	63	F	?	260	50	Ulcerative enteritis
75	64	M	?	340	30	Trauma
76	64	M	?	225	30	Carcinoma of stomach
77	64	M	?	325	40	Carcinoma of bladder
78	65	M	140/80	400	30	Carcinoma of stomach
79	65	M	?	450	20	Luetic aortitis
80	65	M	?	410	30	Trauma
81	66	M	?	425	30	Carbon monoxide poisoning
82	66	M	?	400	30	Dissecting aneurysm of aorta
83	67	M	130/70	320	40	Coronary sclerosis
84	67	M	?	400	50	Carcinoma of rectum
85	67	F	?	370	50	Amebic dysentery
86	69	M	?	400	40	Pneumonia
87	69	M	?	425	30	Bronchopneumonia
88	70	M	Normal	320	40	Astroblastoma
89	71	M	?	300	30	Laryngitis
90	73	M	?	280	40	Carcinoma of pancreas

TABLE I (*continued*)

Case No.	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	yrs.			*gm.*		
91	73	F	?	290	50	Bronchogenic carcinoma
92	73	F	?	340	70	Ruptured appendix
93	73	M	?	450	40	Coronary thrombosis
94	74	F	?	300	50	Peritonitis
95	74	F	148/92	250	20	Cerebral hemorrhage
96	74	M	?	225	70	Trauma
97	75	F	?	250	30	Adenocarcinoma of gall bladder
98	75	F	?	300	30	Generalized arteriosclerosis
99	76	F	?	375	30	Lipoid nephrosis
100	76	F	?	275	40	Bronchopneumonia
101	77	M	?	400	30	Hydronephrosis
102	78	F	?	325	40	Septicemia
103	79	M	?	425	40	Postoperative ileus
104	79	M	?	330	50	Carcinoma of prostate
105	83	M	?	250	60	Hypertrophic pyloric stenosis

outline and stain diffusely. In infants only the reticulum and a few collagenous fibers could be seen in the media. He found a marked increase in collagen in the third decade. By the 40th year there was more collagen than muscle in the media of large arteries. Fibrosis of the arterioles was not described.

Influence of Age: In the small arteries and arterioles of pectoral muscle in my series no instance of marked fibrosis of the media was encountered in an individual under 29 years of age. In one stillborn infant the arterioles contained no fibrous tissue other than the internal elastic lamina and a very fine reticulum surrounding each muscle fiber. Figures 1 and 3 show minimal degrees of fibrosis. After the age of 29 years marked degrees of fibrosis of the media were seen with increasing frequency (see tables).

In Table I the non-hypertensive cases are listed with age, sex, heart weight, the blood pressure when recorded, cause of death, and an estimate in per cent of the amount of blue staining material in the vessel wall when stained with Mallory-Heidenhain stain. The percentage recorded in the tables is an average of estimates of the amount of fibrous tissue in several vessels of the muscle. In Table II the cases of hypertension are similarly tabulated.

TABLE II

Cases With Hypertension. The Degree of Fibrosis of the Media is Estimated

Case No.	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	yrs.			*gm.*		
106	34	F	260/156	470	80	Heart failure
107	36	M	160/106	465	50	Hemorrhage
108	40	F	204/?	400	20	Cerebral hemorrhage
109	43	M	160–240/120	770	40	Heart failure
110	48	F	270/170	682	80	Cerebral hemorrhage
111	49	F	?	575	40	Dissecting aneurysm of aorta
112	51	M	240/?	550	50	Spontaneous rupture of aorta
113	51	F	150/90	275	30	Peritonitis
114	51	M	200/110	450	80	Acute fulminant hypertension
115	54	M	?	575	30	Coronary sclerosis
116	54	M	180/120	650	40	Cerebral hemorrhage?
117	55	M	?	565	50	Uremia
118	55	M	?	575	40	Trauma
119	57	M	170/110	275	40	Congenital cystic kidneys
120	56	F	230/100	350	60	Undetermined
121	58	M	?	580	50	Trauma
122	61	M	?	650	30	Coronary occlusion
123	62	F	155/60	300	30	Peritonitis
124	65	M	?	610	50	Heart failure
125	65	M	?	500	40	Trauma
126	65	M	?	550	30	Trauma
127	65	M	210/100	630	40	Cerebral hemorrhage
128	65	M	?	600	30	Coronary thrombosis
129	70	F	210/90	380	60	Pulmonary embolism
130	70	F	?	450	40	Coronary sclerosis
131	72	F	?	440	40	Undetermined, hypertension
132	73	M	160/80	430	50	Cerebral infarction
133	75	M	160/75	400	30	Bronchopneumonia
134	80	M	190–230/?	400	60	Arteriosclerosis
135	81	M	?	550	30	Pneumonia
136	83	F	170/100	525	50	Cerebral artery thrombosis
137	84	M	?	560	30	Trauma

Figures 2 and 4 show an increase in the degree of fibrosis of the media. A number of cases reached advanced age with only a slight amount of fibrosis. The most marked degree of fibrosis was seen in a 51 year old man who died of acute fulminant hypertension with renal insufficiency. Over 80 per cent of the walls of the arterioles was replaced by fibrous tissue. Only two instances of marked fibrosis were encountered in persons less than 50 years of age. These were

TABLE III

Averaged Estimated Percentage of Fibrous Tissue in Walls of Small Arteries and Arterioles

Cases Without Hypertension

1st decade	10 per cent	1 case
2nd "	30 "	3 "
3rd "	30 "	9 "
4th "	40 "	11 "
5th "	35 "	18 "
6th "	40 "	22 "
7th "	40 "	23 "
8th "	40 "	17 "
9th "	60 "	1 "
Average	38 per cent	105 cases

Cases With Hypertension

4th decade	65 per cent	2 cases
5th "	45 "	4 "
6th "	47 "	10 "
7th "	35 "	7 "
8th "	45 "	5 "
9th "	40 "	4 "
Average	45 per cent	32 cases

both cases of severe hypertension where the individual died with cerebral hemorrhage. Figure 2 shows one of the most marked degrees of fibrosis encountered. However, several cases of hypertension in persons of advanced age showed only slight amounts of fibrosis.

Patients with hypertension have somewhat more fibrosis in the small arteries than do non-hypertensives of the same age group. Table III gives the average percentage of fibrosis in the two groups in the decades of life in which hypertension occurred in this series.

The walls of the small arteries and arterioles that contained relatively large amounts of fibrous tissue appeared to be of about the same thickness as the walls of vessels that contained only small amounts of fibrous tissue. With increase in fibrous tissue there is a loss of muscle. The degree of fibrosis is more or less constant in all the arteries that have a diameter less than 150 microns in the same piece of muscle. The small and large arterioles are involved to about the same degree.

The fibers probably do not arise from the fibrocytes of the ad-

ventitia, as Ssobolew suggested, for no nuclei other than those of the muscle may be seen in the media. They probably arise from the reticulum and the fine collagenous fibers of the media.

Fibrosis of an artery may change its microscopic appearance by decreasing the amount of postmortem contraction. Hesse measured the length of the artery in the arm *in situ* and again after it had been removed from the body. In young persons it contracted as much as 40 per cent in length. In other cases, particularly in older persons, it contracted slightly or not at all.

The treatment of the tissue may also affect the appearance of the arteries. In 1908 MacWilliam and Mackie found that it was possible to stimulate and cause contraction of arteries as much as 30 hours after removal from the body. In lower animals this irritability may last for as long as 4 days. This could be abolished by various methods. Depending on the state of portions of the same artery at the time of fixation, sections showed differences of as much as 25 to 100 per cent in the size of the lumen and as much as 30 per cent in the thickness of the media.

In 1934 Moritz attempted to determine whether or not variations in the technical procedures involved in fixing, dehydrating, and embedding tissues might account for variations in the relative thickness of vessel walls. He found that no significant alterations in the ratios of internal to external diameters could be related to variations in laboratory methods. To learn whether the state of contraction of the vessels at the time of removal from the body was rendered permanent by fixation, samples of skeletal muscles were obtained after administration of vasoconstricting and vasodilating drugs. Neither vasoconstriction nor vasodilatation persisted through the process of preparation of the tissues for microscopic examination. He then concluded that observed differences must be regarded as significant. In a later communication the same author with Oldt reported the histological study of the walls and measurements of the internal and external diameters of over 10,000 arterioles and small arteries from skeletal muscle and the gastro-intestinal tracts of 38 control and 38 hypertensive individuals. Thickening of the walls was characteristic of the hypertensives as a group, but these dimensional changes were not great enough in samples of 75 vessels to permit distinction between hypertensives and controls in 80 per cent of the cases. They stated that they were, however, able to recognize cases

of hypertension and controls as such by the presence or absence of arteriolosclerosis. They interpreted smooth muscle hyperplasia and degenerative changes in the media as arteriolosclerosis. The process not only affected different arteries in the same tissue differently, but in serial sections it was found that different segments of the same artery were affected differently and gave varying wall to lumen ratios.

It should be remembered that a state of contraction of the arteriole may make the vessel appear to have a thickened wall. A rough idea of the degree of contraction may be gained by noting the amount of wrinkling of the internal elastic lamina. By applying varying amounts of tension on the walls of aortas Zon found that the internal elastic membrane became straight at pressures less than diastolic blood pressure. With the application of more weight the elastic membrane stretched like a rubber band.

The wall to lumen ratios of many small arteries and arterioles were determined. There is a correlation between the degree of contraction of the vessel and the wall to lumen ratio. There is a higher wall to lumen ratio in those vessels in which the internal elastic lamina is tortuous. The ratios were so obviously influenced by the state of contraction of the vessel, as seen by the length of the internal elastic lamina, that attempts to measure the vessels accurately were abandoned. The relation between the state of contraction of the artery and the wall to lumen ratio was the only constant relation observed.

Figure 4 shows two arterioles in the same section of pectoral muscle. In one the internal elastic lamina is thrown into folds and the vessel appears to have a thick wall. The elastic lamina of the other arteriole is fairly regular. This vessel appears to have a thin wall. Because of the manner in which the adventitia of many of the arterioles of pectoral muscle blends with the surrounding connective tissue, accurate measurements of the outer margins may be difficult or impossible. Figures 3 and 4 illustrate this source of error.

In the same section of muscle arterioles may show different degrees of contraction and wide variations in wall to lumen ratios. Unless the amount of contraction can be judged, the relative thickness of the vessel wall cannot be determined.

Summary and Conclusions

In a study of specimens of pectoral muscle from 137 individuals a marked variation in the degree of fibrosis of the media was observed. This may be seen in sections stained with the Mallory-Heidenhain stain.

The degree of fibrosis was most marked in cases of severe hypertension. There was a somewhat greater average amount of fibrous tissue in the walls of the arterioles in hypertensives than in non-hypertensives of the same age group. All cases of hypertension did not show marked fibrosis.

No cases of marked fibrosis were seen before the 29th year of life. After that age it was encountered with increasing frequency. However, many persons of advanced age show only minimal degrees of fibrosis.

The connective tissue fibers apparently arise from the reticulum and collagenous fibers in the media.

The wall to lumen ratio is determined by the contraction of the vessel which can be estimated by the length and tortuosity of the internal elastic lamina.

No intimal disease was found in the small arteries or arterioles of pectoral muscle at any age. It was not seen in any case of hypertension.

It was not possible to distinguish between hypertensive and control patients by examination of the small arteries and arterioles of pectoral muscle.

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DESCRIPTION OF PLATE

PLATE 119

FIG. 1. Case 116. Male, 54 years of age. Blood pressure 180/120. Heart weight 650 gm. Left ventricular hypertrophy. Death due to cerebral hemorrhage (?).

Arteriole with an outside diameter of 32 microns. A fine layer of reticulum surrounds each smooth muscle cell. Low degree of fibrosis. Mallory-Heidenhain stain. × 600.

FIG. 2. Case 106. Female, 34 years of age. Hypertension discovered 1 year before death. Blood pressure 260/156. Death due to acute heart failure. Heart weight 470 gm.

Arteriole with an outside diameter of 78 microns. There is marked fibrosis of the vessel wall. The persistent muscle fibers are seen between the collagenous fibers. Mallory-Heidenhain stain. × 600.

FIG. 3. Case 135. Male, 81 years of age. Heart weight 550 gm. Left ventricular hypertrophy. Blood pressure not known. Diabetes mellitus and lobar pneumonia.

Arteriole with an outside diameter of 40 microns in a relaxed state. Note absence of tortuosity of internal elastic lamina. Wall to lumen ratio 1.0:5.0. Minimal degree of fibrosis. Mallory-Heidenhain stain. × 900.

FIG. 4. Case 77. Male, 64 years of age. Heart weight 325 gm. Death from carcinoma of urinary bladder.

Two arterioles from the same section of pectoral muscle with outside diameters of 85 and 82 microns respectively. The walls of the two vessels are in different states of contraction. The artery in the lower right corner is contracted as is shown by its wavy internal elastic lamina. Its wall to lumen ratio is 1.0:2.0. The artery in the upper left corner is relaxed as is shown by its straight internal elastic lamina. Its wall to lumen ratio is 1.0:4.0. Mallory-Heidenhain stain. × 180.

1

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Andrus

Structure of Arteries and Arterioles

MICROTECHNICAL DEMONSTRATION OF IRON *

A CRITICISM OF ITS METHODS

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Iron contained in the tissues occurs in two forms. In one group of compounds, generally included under the denomination "hemosiderin," the iron reacts similarly to any inorganic, insoluble iron compound, readily demonstrable by the well known iron reactions of analytic chemistry. In the second group of compounds iron can be demonstrated only after thorough chemical destruction, *e.g.* incineration. In the latter group belong, among others, hemoglobin, methemoglobin and hematin. In this paper I wish to deal with the demonstration of hemosiderin-iron only.

There are three methods and their modifications, respectively, known for the demonstration of iron in microscopic preparations: (1) the Berlin blue method of Perls; (2) the iron sulphide reaction of Quincke (described by Mayer as early as 1850); and (3) the Turnbull blue method of Tirmann and Schmelzer. This last method is based on Quincke's reaction. According to the Berlin blue method of Perls the microscopic section is treated with hydrochloric acid and potassium ferrocyanide. Iron dissolved from hemosiderin by the hydrochloric acid reacts with the potassium ferrocyanide, producing a precipitate of Berlin blue. According to the iron sulphide reaction of Quincke, iron compounds are converted by yellow ammonium sulphide into iron sulphide. On account of the energetic reducing properties of this reagent, ferrous sulphide is formed from both ferrous and ferric compounds. Ferrous sulphide is highly sensitive to oxygen and acids, and preparations will deteriorate within a few days in spite of all possible care. Therefore, the Turnbull blue method of Tirmann and Schmelzer transforms ferrous sulphide by means of an acidulated solution of potassium ferricyanide into Turnbull's blue.

The question as to which of the methods mentioned is the best to

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demonstrate the greatest amount of iron present and to give the truest picture has been rather extensively debated. Generally speaking there are but a few adherents of the Berlin blue method (Zaleski, Arnold and Sumita). According to Sumita no method is superior to that of the Berlin blue. He reproduces a table of Neumann's showing that colorimetric demonstration of ferri-iron as Berlin blue is about 250 times more sensitive than its demonstration as iron sulphide. On the contrary, the great majority of workers on this topic are of the opinion that the iron sulphide reaction and Turnbull's blue method are more sensitive, demonstrating a greater amount of iron than the Berlin blue method. According to Quincke, the details, as revealed by the iron sulphide reaction, are much finer than those by the Berlin blue method. In his opinion this is due to the fact that iron is present in the tissues partly in the form of ferrous compounds which do not react with potassium ferrocyanide. Moreover, he supports the greater sensitivity of the iron sulphide reaction with the observation that egg yolk is stained dark green by ammonium sulphide, whereas if treated with hydrochloric acid and potassium ferrocyanide it becomes but pale blue. He also contends that the Berlin blue method will occasionally produce false positive reactions in the absence of iron compounds. According to Hueck, Turnbull's blue method is more reliable, especially if small amounts of iron are to be demonstrated. He is unable to explain this, as he never could demonstrate ferrous compounds in the tissues with the direct Turnbull's blue reaction. He observed that potassium sulphocyanate demonstrates a conspicuously smaller number of granules than ammonium sulphide. Nishimura's modification of the Tirmann-Schmelzer method consists of employing potassium ferrocyanide instead of ferricyanide after previous treatment with ammonium sulphide by reason of quick oxidation of the white ferro-ferrocyanide formed at first into ferri-ferrocyanide (Berlin blue) by the oxygen of the air. He declares that he was often able to prove the presence of ferrous in addition to ferric compounds in tissue sections. In his opinion the alleged higher sensitivity of Turnbull's blue method is due partly to the fact that it demonstrates both ferric and ferrous compounds. Mallory, too, prefers modifications of Turnbull's blue to the Berlin blue method, partly on the basis of the theory just mentioned, partly because Berlin blue is soluble in an excess of potassium ferrocyanide, causing blurring of the picture. Lubarsch also recommends Turn-

bull's blue method as the most sensitive of all. In short, according to the generally accepted opinion in the medical literature on this question, Turnbull's blue is the most dependable, demonstrating the greatest amount of iron: this tenet is accepted by all textbooks of microtechnique.

In the course of the year I performed the iron reaction on a great number of microscopic preparations and made observations contrary to the general view mentioned. Therefore I set out to reinvestigate the entire question of the microtechnical demonstration of iron. My material was rather miscellaneous: formalin and formalin-alcohol fixed organs from cases of pernicious anemia, organized hematomas of various ages, a case of pigmented giant celled xanthoma and the organs of a rabbit dying of phenylhydrazine intoxication. Many microscopic sections were prepared from all tissue blocks and all types of iron demonstration, as found in the literature, were tried on them, together with some methods devised by me. My results were as follows:

1. I was unable to demonstrate even traces of ferrous compounds in any material with the direct Turnbull's blue reaction. Therefore I see no reason why the Stoeltzner method, using a mixture of potassium ferro- and ferricyanide in order to ensure demonstration of both ferric and ferrous compounds, should ever be employed.

2. Different modifications of the Berlin blue method are by no means of the same value. Those modifications using hydrochloric acid and potassium ferrocyanide separately will invariably show a smaller amount of iron than those using them simultaneously. Those using acid first (method of Stieda) are fundamentally wrong, since the iron dissolved by the acid before the application of potassium ferrocyanide is irretrievably lost. But even those using the reagents in reverse order are objectionable. This is but natural. Ferric chloride is but slowly split from hemosiderin by hydrochloric acid; in the meantime almost all the potassium ferrocyanide taken up by the section during previous treatment with this substance will diffuse away and become so highly diluted in the excess of hydrochloric acid that its concentration will become entirely insufficient. This applies even to the method of Sumita (incubating the sections with a concentrated solution of potassium ferrocyanide). In this way both theoretically and practically only those methods are justifiable that use both reagents simultaneously. But even in the

latter case too short a time of exposure may constitute a serious source of error. Even when using a 10 per cent solution of hydrochloric acid (commercial pure concentrated hydrochloric acid, conchloric about 40 per cent of hydrochloric acid gas by weight, being taken here for 100 per cent) it can be easily observed that for about 20 to 25 minutes the number of blue granules will steadily increase, whereas after 25 minutes no more new granules are noticed. If less concentrated solutions are used the completion of the reaction, of course, will take an even longer time. This has been observed also by Nishimura. The concentration of the potassium ferrocyanide seems to be less important: a 1 to 2 per cent solution is quite sufficient. However, in order to prevent the Berlin blue precipitate from dissolving it is necessary to use a more concentrated solution (this point will be dealt with once more under Step 3). On the basis of experience I suggest the use of a mixture made up of equal parts of a 10 per cent potassium ferrocyanide and a 20 per cent hydrochloric acid solution, prepared freshly with distilled water and filtered. The time of exposure should be 30 minutes. This mixture has a satisfactory stability; it begins to show a greenish tinge after about 45 minutes, but no precipitate is formed even after several hours. Even so, it is advisable to place the slide with the section side down in order to avoid the deposition of any possible precipitate. Berlin blue will not be damaged by the acid as it is almost insoluble in dilute acids; by alkalis, however, it will be bleached. Therefore, counterstaining with lithium carmine is not advisable. For nuclear staining I used Kernechtrot (a red nuclear stain obtained from the German firm, Hollborn), with invariably satisfactory results. The method outlined yields an extraordinarily sharp and complete iron reaction, equal to the iron sulphide but without its drawbacks and decidedly superior to the Tirmann-Schmelzer method and any of its modifications. This was especially conspicuous in cases where minute amounts of iron had to be demonstrated. I did not observe a single case in which Turnbull's blue method gave a critically acceptable evidence of a greater amount of iron than the method described.

3. That in spite of the facts mentioned Turnbull's blue is considered the most reliable method by the majority of workers on this problem is due to several reasons, no one of which, however, can stand objective criticism. First, there are theoretical assumptions

which cannot be substantiated in practice, *e.g.* the presence of ferrous compounds. Hueck's statement that a conspicuously small number of granules react with potassium sulphocyanate is by no means a proof since iron sulphocyanate is easily soluble and its rapid diffusion renders its exact observation impossible. It is interesting that Nishimura, who also uses potassium ferrocyanide when performing the Tirmann-Schmelzer reaction on the basis of the rapid transformation of white ferro-ferrocyanide into Berlin blue, accepts as a possible cause of the alleged lower sensitivity of the Berlin blue method the fact that it fails to demonstrate both ferric and ferrous compounds; the obvious contradiction between his two statements seems to have escaped his attention. Moreover, as has been shown, hemosiderin does not contain ferrous compounds. In the egg yolk test of Quincke the darker color produced with the iron sulphide reaction is not a proof of higher sensitivity but is due simply to the fact that the staining power of ferrous sulphide is greater than that of Berlin blue. This can easily be shown by the following simple test: put one drop each of ammonium sulphide and of a mixture of sodium ferrocyanide and hydrochloric acid on a blotting paper moistened with a highly diluted (0.1 per cent) solution of ferrous sulphate: the spot caused by the first reagent will be dark green, almost black, whereas that caused by the second reagent will be much lighter blue, although both reactions may be considered quantitative. The objection of Mallory to the Berlin blue method is that Berlin blue is soluble in an excess of potassium ferrocyanide, which causes a diffuseness of the staining. I at once state that I never observed blurring attributable to Berlin blue itself; moreover, it can be easily shown that the assertion of Mallory does not hold. Prepare an aqueous suspension of Berlin blue, divide it into four equal parts, dilute these portions to equal volumes with (1) distilled water, (2) 10 per cent hydrochloric acid, (3) a 5 per cent solution of potassium ferrocyanide, and (4) a mixture of the two last solutions. Centrifuge for several minutes and observe the supernatant fluids. In (1) it will be dark blue, in (2) pale blue, and in (3) and (4) pale greenish yellow. This shows clearly that potassium ferrocyanide strongly reduces the solubility of Berlin blue. If the Berlin blue and Turnbull's blue methods are performed on microscopic preparations of the same material, the preparations treated according to Turnbull's blue method often will look without question more blue. If the sections

are compared under the microscope it will be noticed that with the Berlin blue the blue granules are tiny, dense, homogeneous and sharply outlined, similar to the granules of eosinophilic leukocytes. No other morphological element but round granules can be seen. On the contrary, in sections treated according to Turnbull's blue method the blue granules are of a much larger size (this has been noticed by Tirmann, who erroneously attributed this phenomenon to the greater sensitivity of his method); very often they have darker outlines and a paler center; not rarely they are surrounded by a more or less extensive pale blue halo. In addition, from larger granules very often bizarre, whorly or twig-like processes (also with darker outlines) take their origin. These peculiarities observed with Turnbull's blue method are the more marked the more acid is used. If the mixture suggested by the originators (equal parts of 1 per cent hydrochloric acid and a 20 per cent potassium ferricyanide solution) is used, they are quite conspicuous, but are much less so if only one drop of hydrochloric or, even better, acetic acid is added to each 50 cc. of the ferricyanide solution. The section, however, does not become blue as rapidly. If both sections are compared with a third one, made according to the method of Quincke (in which a 1 hour exposure to ammonium sulphide has proved amply sufficient), it will be noticed that the morphology of the iron, according to the Berlin blue method and the iron sulphide reaction, is absolutely identical as regards the number as well as size and shape of the granules; whereas the formations observed with Turnbull's blue are not duplicated by any other method. The cause of this interesting fact is the following: with the Berlin blue method ferric chloride is but slowly split from hemosiderin and bound at once, locally, by potassium ferrocyanide. On the contrary, in the case of Turnbull's blue method, hydrochloric acid dissolves iron sulphide almost with the vehemence of an explosion, but anyhow at a rate greatly surpassing the reaction speed of the formation of Turnbull's blue, the latter being retarded also by a semipermeable membrane of its own substance formed on the surface of the granules. In addition, particles are torn and carried off by gas bubbles. The curious artifacts described are formed in this way, as can easily be observed if the conversion in sections of ferrous sulphide into Turnbull's blue is examined under the microscope. Of course, if, following the suggestion of Mallory, no acid is used these faults will not occur, but the transformation of

ferrous sulphide into Turnbull's blue will be an incomplete one, and in addition many granules will acquire a dingy greenish color, rapidly fading out under the coverslip. Another method devised by Mallory, using 5 per cent acetic acid, is in no respect superior to the original one. Should someone, convinced of the superiority of the iron sulphide reaction, prefer the same, I would advise him to render his preparations durable, not by the Turnbull blue method, but rather by converting ferrous sulphide into either copper or lead sulphide. This can easily be accomplished by washing the section (treated previously with ammonium sulphide) first with pure diluted ammonia, then with distilled water and placing it for 1 hour in either a 5 per cent copper sulphate or lead nitrate solution. The deep black granules of copper or lead sulphide will give in sections counterstained with methylene blue or any red nuclear stain a beautiful picture. Hematoxylin is not recommended for counterstaining. The granules will be but slightly larger than those of the original ferrous sulphide, the difference being insignificant.

While working on this subject I was much annoyed by the fact that both the Berlin blue and Turnbull's blue preparations soon began to lose their color and within a few months, or even weeks, became entirely unsuitable for comparison. This phenomenon is well known and was described by Gans in 1923. Although the preparations can be restored by removing the coverslip, I tried to find a method by which the sections could be made permanent. My experiments were based on the following idea: fading out of Berlin blue or Turnbull's blue sections is quite different in nature from that of hematoxylin or methylene blue. In a section stained with hematoxylin, fading starts from the margins and is progressive toward the center; the process is irreversible. It is probably caused by oxidation. Decolorization of the methylene blue stain can be almost completely prevented by the use of acid-free balsam; therefore it is to be attributed to an acid reaction. Fading out of an iron reaction invariably starts at the center and if the section is not much smaller than the coverslip, its peripheral parts will not lose their color. The decolorization cannot be retarded by using neutral balsam. These facts, together with the quick restoration of the preparations if exposed to air or, even better, to a diluted hydrogen peroxide solution, point to the decolorization being due to reduction. Canada balsam, too, like resins in general, probably takes up oxygen while

drying, depriving the section of its oxygen in this way and reducing both Berlin blue and Turnbull's blue to colorless ferro-ferrocyanide. On this basis it seemed that a mounting medium rich in oxygen would prevent or at least retard the fading out of sections. This idea proved to be sound. I diluted inspissated, almost dry Canada balsam with old, resinified oil of turpentine (known to contain peroxides) until it became of syrup-like consistence and tried to mount sections with it. It turned out that all sections mounted with this medium were much more durable than those mounted with simple Canada balsam. On account of the shortness of time that had elapsed since my experiments (5 months) I am unable to state the exact duration of sections preserved in this way. At any rate, all my preparations mounted with Canada balsam almost completely faded out in this time, whereas those mounted with the new medium remain unchanged. Canada balsam preparations all faded out within less than 2 days if kept at 56° C., whereas those preserved with oil of turpentine completely withstood this temperature for more than 14 days. Oil of turpentine does not destroy either carmine or Kernechtrot stain. I tried also to dilute Canada balsam with a solution of benzoyl peroxide in xylene; this method, however, proved to be much inferior to the first one. Therefore, I recommend the mounting of iron reaction preparations with Canada balsam diluted with old, resinified oil of turpentine.

Summary and Conclusions

1. Hemosiderin does not contain ferrous compounds demonstrable with the direct Turnbull's blue reaction.

2. The alleged superiority of the Tirmann-Schmelzer modification of Turnbull's blue method is based partly on erroneous theoretical conceptions and partly on the misinterpretation of artifacts.

3. The best microtechnical reagent for iron is a mixture of equal parts of 20 per cent hydrochloric acid and a 10 per cent solution of potassium ferrocyanide. The exposure should be 30 minutes. Results of equal quality are produced by converting ferrous sulphide obtained in Quincke's reaction into copper or lead sulphide.

4. Both Berlin blue and Turnbull's blue preparations can be made durable by diluting the Canada balsam to be used in mounting sections with old, oxidized oil of turpentine.

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DESCRIPTION OF PLATE

PLATE 120

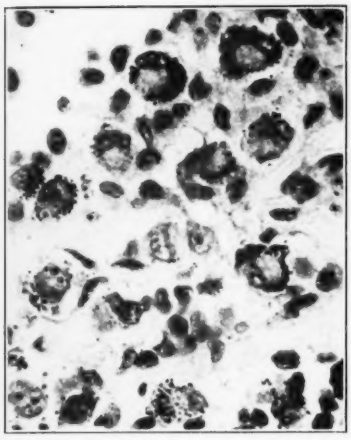
All photomicrographs were made of the same material (pigmented giant celled xanthoma of a tendon sheath) under identical optical conditions. Hematoxylin nuclear stain.

FIG. 1. Author's modification of the Berlin blue method. × 300.

FIG. 2. Author's modification of the Tirmann-Schmelzer method (conversion of ferrous sulphide into lead sulphide). × 300.

FIG. 3. Turnbull's blue method. At "A" granules appear as tiny ringlets (diffusion artifact). × 300.

FIG. 4. Typical artifact (twig-like processes) as produced with the Turnbull's blue method. × 300.



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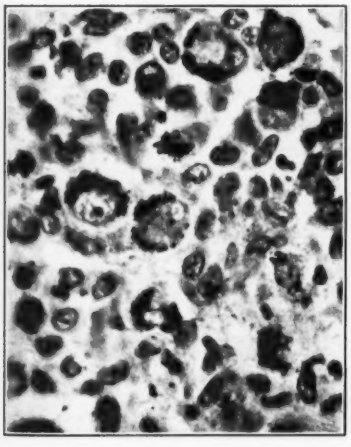

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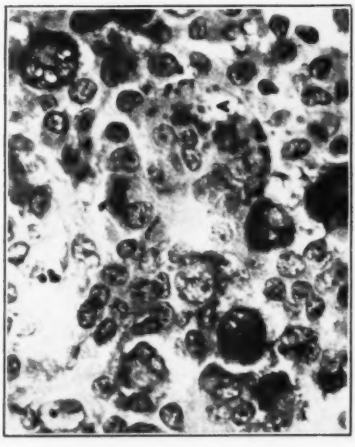

3

Gömöri Microtechnical Demonstration of Iron

THE REGENERATION OF AUTOPLASTIC SPLENIC TRANSPLANTS *

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The first successful autotransplantation of splenic tissue in animals was made by Marine and Manley.[1] They found that subcutaneous or intramuscular splenic transplants in the rabbit were successful in almost 100 per cent of instances, and that immaturity of the animal was a powerful stimulus to the growth of the transplants. In immature rabbits with one-eighth of the spleen removed there was a slight growth of the transplant which increased rapidly following subsequent total splenectomy. They believed that this stimulus to growth was chemical in nature, because the grafts were removed from their normal neurovascular supply. The histology of the transplants was identical in all characteristics with normal spleen tissue. Spleen grafts, if made in immature rabbits accompanied by complete splenectomy, were permanent throughout the life of the animal. They further observed that although spleen transplants in rabbits were successful, in the absence of a physiological deficiency of spleen tissue they soon atrophied. When there was a definite splenic deficiency due to total splenectomy, autotransplants were not only successful but they grew and increased in size. In mature rabbits, whether splenectomized or not, the transplants regenerated but were slowly resorbed. From their observations they concluded that in the rabbit the spleen is most important in early life. After sexual maturity it is either unimportant or its function may readily be assumed by other hematopoietic tissues.

Subsequently Perla and Marmorston-Gottesman observed the regeneration of autoplastic splenic transplants in the rat.[2, 3] They found that such transplants in the adult rat regenerated and grew in over 90 per cent of instances, even in the presence of the spleen. In many instances it was further observed that the regenerated transplants may function in place of the normal spleen. As evidence of this it was found that in a strain of rats that developed *Bartonella muris* anemia after splenectomy, autoplastic splenic transplants, performed 7 weeks prior to splenectomy, afforded protection against

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this disease in more than 50 per cent of instances. A conspicuous difference was noted in the histology of the transplants that protected and those that did not protect the animal. In the protected rats the red pulp appeared normal and showed a large amount of phagocytosis of iron-containing pigment by the reticular cells and macrophages. In the transplants of the animals that died of *Bartonella muris* anemia only a few lymphoid cells were found in the red pulp. Many of the nuclei of the reticular cells showed evidence of necrosis. Only minute amounts of iron-containing pigment were found in the macrophages. The sinuses of the red pulp were engorged with blood. The follicles were sharply defined and showed no pathological change.

In the present communication the detailed histogenesis of autoplastic splenic transplants is reported.

Methods *

In this work 24 mature albino rats free from *Bartonella muris* infection (Wistar stock), about 3 months of age, were used. The spleen was exposed through a small incision in the abdominal wall and one pole was gently pulled up into the opening. A piece was cut off for transplantation. Pieces of spleen about 6 mm. in diameter were immediately placed in small prepared pockets in the abdominal wall. Great care was exercised to avoid hemorrhage into these pockets, and if hemorrhage occurred a new pocket was made. The sites of the transplants were indicated by closing the mouth of the pocket with a black silk suture. The method of transplantation was essentially the same as that employed in the transplantation of thymus and lymph node tissue reported in previous communications by Marmorston-Gottesman and Jaffe.[4,5] Four transplants were usually inserted in each animal. Two rats each were killed at intervals of 2, 18, 24 and 36 hours, and 3, 4, 5, 6, 7, 12, 21 days and 7 weeks after operation. The transplants were carefully removed, fixed in Bouin's fluid, and embedded in paraffin. Sections were stained with hematoxylin-eosin, Verhoeff's elastic tissue stain, Pacini's stain, and by Bielschowsky's silver impregnation method.

* I am indebted to Dr. Jessie Marmorston for surgical assistance in the transplantations.

Presentation of Data

This study is based on an examination of 96 splenic transplants. A description of the successive stages of degeneration, regeneration and growth of the transplants follows.

2 Hours: Only at the cut edge of the splenic transplant is there evidence of necrosis in the splenic tissue. In the remaining tissue the cells are intact, the architecture clear. In the muscle surrounding the transplant there is little cellular reaction to its presence. The cytoplasm of the surrounding muscle fibers is swollen and shows granular degeneration.

15 Hours: There is evidence of necrosis throughout the entire transplant except for a thin outer zone of viable tissue. This zone shows well formed lymphocytes and reticular cells. The rest of the transplant architecture is poorly defined. The nuclear elements show various stages of karyorrhexis. In some sections extensive hemorrhage is present in and about the transplant. The surrounding tissue contains many polymorphonuclear leukocytes, very few mononuclear phagocytes but a considerable amount of edematous fluid.

24 Hours: The transplant is infiltrated with blood. There is an extensive necrosis of the entire splenic tissue except for a narrow zone where a layer of well defined cells is present, at the outer margin of the transplant. Fragments of nuclear elements are present throughout. The surrounding tissue is edematous and is infiltrated with many polymorphonuclear leukocytes and a few mononuclear phagocytes.

36 Hours: In some areas a portion of the splenic capsule is still preserved, and just beneath the capsule within the transplant reticular cells, lymphocytes and well formed sinuses are seen. There are small areas of hemorrhage and erythrophagocytosis by macrophages is seen. There is a zone of apparently viable reticular cells at the periphery which merges with the necrotic tissue that occupies the rest of the transplant. In many areas the entire architecture of the splenic tissue is destroyed. Nuclear fragments, cellular débris and many red blood cells and polymorphonuclear leukocytes are present within the transplant. In these areas lymphocytes and reticular and endothelial cells in all stages of degeneration are visible. The

blood vessels are included in the massive necrosis which is seen in the transplant.

The reticulum and collagen fibrils within the area of necrosis are not distinguishable. A few elastic fibers are still present in the connective tissue septa of the transplant. The remnant of capsule around it contains some elastic tissue. That of the small arterioles throughout the transplant is destroyed.

Around the periphery of the transplant the tissue is edematous, and phagocytic macrophages, many of which have engulfed débris and red blood cells, are present in large numbers. Occasional polymorphonuclear leukocytes are seen and a zone of young fibroblasts surrounds the transplant.

48 Hours: In the central portions of the transplant the splenic tissue is destroyed. The site of the malpighian corpuscles is discernible but the cells show all stages of degeneration. Throughout most of this area the reticular and endothelial cells are necrotic. In many places they are swollen and show evidence of erythrophagocytosis.

At the periphery of the transplant and beneath the remnant of the capsule numerous reticular-like elements are present. These appear to be growing into the transplant (see Fig. 1). The cytoplasm of these cells forms an irregular syncytium. Red cells are present in ill defined vascular spaces in this zone, but few lymphocytes are seen. The reticulum of the capsule of the transplant is sharply defined and in the tissue surrounding the latter the capillaries are congested, areas of hemorrhage are seen, and many polymorphonuclear leukocytes and phagocytic macrophages containing ingested red blood cells are present.

3 Days: In larger transplants the outer half is completely replaced by a mesh of syncytial reticular cells which surrounds and invades the central area of necrotic tissue with finger-like projections.

In one small transplant the reticular cells have regenerated and have almost completely overgrown the transplant. It is completely replaced by what appear to be reticular cells (see Fig. 2). There are few lymphocytes present. The fibrillar and cellular reticulum has formed a mesh of sinuses which gives it a honeycomb appearance. These sinuses are lined with flattened endothelium and

in places it is apparently proliferating and large cells lie free in the sinuses. In other areas several of these cells have fused to form irregular multinuclear giant cells. In reticular and endothelial cells the cytoplasm is scanty and vacuolated. The nuclei are large, pale and vesiculated, and contain finely scattered chromatin material. The cytoplasmic processes anastomose with those of surrounding cells. The fibrillar reticulum can be traced about the sinuses, weaving an interlacing mesh throughout the transplant.

In larger transplants the formation of sinuses has not as yet occurred, although capillaries containing blood cells are present.

4 Days: A wide outer zone of the transplant shows large numbers of reticular cells, some of which contain mitotic figures. Their cytoplasm forms a loose syncytial mass penetrating the inner portions of the transplant. Some of these cells contain iron pigment. There are no lymphocytes in this outer zone but there are many red blood cells which lie in irregular, poorly defined sinusoidal spaces. Elliptical endothelial cells, apparently arising from the reticular cells, line the irregular blood spaces in some places. The formation of new fibrillar reticulum forming primitive sinuses within the outer zone can be distinguished. In one area a large mass of reticular cells is arranged in a concentrically lamellated whorl about a capillary space and a few elements resembling small lymphocytes are found in the interstices of this tissue. The reticular fibrils penetrate the outer portion of what appears to be a primitive follicle (see Fig. 3).

In the center of the transplant there is a small area of necrosis, surrounded by a broad zone of mononuclear phagocytes, containing nuclear débris, red blood cells and hemosiderin. Numerous fibroblasts are seen about the transplant and in some areas they seem to invade it. This does not occur in the zones covered by transplanted splenic capsule. The connective tissue of the capsule and the reticulum in the outer zone of the plant is clearly defined. Extending from the capsule are connective tissue septa in the process of formation.

5 Days: There is still a central area of necrosis with a zone of hemorrhage. The outer half of the transplant shows complete regeneration. A partly developed follicle close to an arteriole can be seen. All stages of lymphoid cell formation are visible. The reticular cells

vary considerably in size and shape, and transition forms between large, oval reticular cells and fully developed lymphocytes may be distinguished.

The reticular fibrils in the regenerated part of the transplant form a fine pattern of sinuses. In one area a prominent and well formed arteriole lies in the center of a follicle. The elastica of the wall is fully developed.

The connective tissue of the capsule is clearly defined. The connective tissue septa are present, extending into the transplant. Scattered outside of its capsule are reticular cells, lymphocytes, occasional polymorphonuclear leukocytes and mononuclear macrophages containing blood pigment.

6 Days: The transplant presents a remarkable picture. There is a small central area of necrosis. Within this area are ghosts of large cells containing blood pigment but showing evidence of degeneration. The necrotic zone merges imperceptibly with a zone of loosely arranged, large mononuclear cells containing large amounts of blood pigment. These large reticular-like elements gradually become more compact as the outer portion of the transplant is approached. An occasional well formed arteriole is seen in this area. The outer two thirds of the transplant show fully regenerated splenic tissue. Sinuses containing red blood cells are present. Reticular and endothelial cells are rich in blood pigment. Lymphocytes, although scattered through the pulp, accumulate more prominently in some areas where follicle formation is apparently occurring. Cells suggesting transition forms between large oval reticular cells and fully developed lymphocytes may be distinguished.

Within a primitive follicle a small arteriole seems to be in the process of formation. The outer portion is composed of whorls of flattened reticular-like cells with elliptical nuclei. The inner portion of the structure consists of a syncytium of large, pale, reticular elements with pale staining cytoplasm. The lumen is not definitely seen. A well defined zone of fibrillar reticulum is discernible in the outer portion of the wall. Another arteriole shows a later stage with more definite lumen formation. The connective tissue of the media is more deeply stained and condensed. The lumen contains red blood cells and is lined with a syncytium of large, vesiculated endothelial cells. The transplant has a well defined capsule which sharply separates it from the surrounding tissue. Surrounding the

transplant, outside of the capsule, is a fibrillar connective tissue structure containing macrophages rich in blood pigment. In one area in direct contiguity with the capsule, but outside of it, there is a large cluster of reticular cells arranged in a concentric whorl showing evidence of follicle formation and containing various transition forms between reticular cells and lymphocytes.

7 Days: There is no area of central necrosis. The entire inner two-thirds of the transplant is completely replaced by a loose syncytium of reticular cells. Many of these cells in this zone and in the outer zone contain a large quantity of blood pigment. In the outer third of the transplant, in addition to the syncytial mass of reticular elements, there are several well defined follicles with lymphocyte formation. Cells suggesting all stages of lymphoid cell formation from large reticular cells to lymphocytes are present. An occasional normoblast may be seen. A large arteriole in the process of formation is evident in one area. The lumen is lined by flattened endothelial cells and about this is a loosely arranged whorl of reticular cells.

The sinuses in the outer zone are not so clearly defined as in some of the other transplants, but where they occur they are markedly distended with blood. The capsule is composed of dense collagen fibrils and well defined trabeculae extend from the capsule into the transplant.

12 Days: The transplant is replaced by large numbers of reticular cells. Sinuses containing blood elements are distinguishable . In the outer third are scattered lymphocytes. Veins and arterioles are present. In one area close to a newly formed arteriole there is a clump of modified reticular cells which are round and vesiculated, but their nuclei contain more condensed chromatin masses than are found in reticular cells. They appear to be lymphoblastic cells. Cellular elements suggesting gradations from these to adult lymphocytes may be distinguished. Many pigment-laden mononuclear macrophages are present in the transplant. It is demarcated by a definite capsule containing collagen fibers. The surrounding tissue contains a few polymorphonuclear leukocytes and large macrophages laden with iron pigment.

21 Days: The transplant shows evidence of complete regeneration with the formation of a capsule and trabeculae. Foci of lymphocytes surround the arterioles and suggest follicle formation. Well formed

sinuses are present and these are congested. There is evidence of erythrophagocytosis by macrophages. Occasional megakaryocytes are seen in the red pulp.

7 Weeks: The transplant is a small spleen about 1.5 cm. in diameter. The elements are completely differentiated. The splenic follicles and the blood sinuses, with the normal red pulp elements, including megakaryocytes, are present. Blood pigment is present within the macrophages and reticular and endothelial cells (see Fig. 4).

Discussion

The phylogenetic and ontogenetic development of the spleen may be simulated in the adult rat in the regeneration of autoplastic transplants of fragments of splenic tissue within a few days. The observations reported in this communication suggest that the reticular cells of the adult spleen retain their potentiality for differentiation and that they may be the precursor of the structural cellular elements of the spleen. These studies further suggest that the lymphoblastic tissue may arise from the reticular cell.

Phylogenetically it appears that the entire pulp of the spleen (red and white) develops from a common ancestral mesenchymal reticulum. In certain lower vertebrates, as in fish and amphibia, there is no distinction between the malpighian corpuscles and the red pulp. In the selachians, reptiles and birds, the lymphatic follicles become prominent and stand out against the vascular pulp, but masses of lymphocytes are scattered throughout the red pulp(Klemperer [6]). In the human, the spleen arises embryologically in the dorsal mesogastrium from a group of round cells which appear cytologically identical with those of the surrounding mesenchyme but are more compact. During the early phase of its development the undifferentiated mesenchyme increases in amount and forms a rich vascular network. Later the pulp reticulum, venous sinuses, arterial sheaths and lymphatic tissue develop. At 8 weeks the mesenchymal cells are surrounded by delicate fibers (Ono [7]). In the 3rd month a capsule and trabeculae develop. During the 5th month a vascular bed develops by separation, loosening and rearrangement of the reticular cells. The venous sinuses consist of delicate endothelial-lined tubes apparently continuous with the primary embryonal plexus of veins. At this time hematopoiesis is observed in the growing spleen.

Hemocytoblasts separate from the fixed mesenchymal cells. These differentiate into erythroblasts. After the 5th month this process decreases and is practically absent in the 6th month (Ono).

According to Ono, the development of the lymphatic tissue is associated with the formation of the arterial system. When the branches of the splenic artery acquire a muscular wall during the 4th month, they become surrounded by a mantle of reticular tissue. Early in the 5th month within these areas accumulations of lymphoid cells appear which separate themselves from the surrounding reticular cells. The follicles develop during the 5th and 6th month within the angles of the arterial tree. The vascular network of the follicles is fully developed at the end of fetal life.

From the study reported in the present communication it is evident that the histogenesis of the splenic elements in the regeneration of the autoplastic transplants repeats the ontogenetic development of the spleen and suggests that the major elements of this organ have a common origin in the mesenchymal reticulum.

It is striking that the developmental potentiality of the reticular cell is retained in adult life in the rat and that regeneration of splenic autotransplants readily occurs even in the presence of most of the spleen.

A comparison of the regeneration of thymus autoplastic transplants [4] and of lymph node transplants [5] with that of splenic tissue reveals the fact that the reticular cell in each case is the common ancestral cell of the structural elements subsequently found in the fully regenerated tissue. Morphologically the reticular cells of the lymph node, thymus and spleen transplants which survive in the extreme outer zone are apparently identical, and are closely related in their capacity to form lymphoid tissue and in the property of phagocytosis. But in each instance they must possess some specific growth trend different from the reticular elements of other organs.

Summary and Conclusion

The histogenetic development of the regeneration of autoplastic splenic transplants was studied in a series of adult albino rats. It was found that the transplanted splenic tissue undergoes rapid degeneration within the first 24 hours. A thin zone of viable reticular cells survives. There is little inflammatory reaction about the

transplant. By the 3rd day there is marked proliferation of the reticular cells at the outer zone and streamers of cells are seen penetrating the necrotic center of the transplant. In some instances the entire transplant is replaced by reticular cells which have formed the pattern of a sinus organ. By the 3rd or 4th day lymphocytes appear irregularly in clusters and apparently all stages of lymphoid cell formation are observed.

By the 6th day the outer two-thirds of the transplant are replaced by reticular elements arranged in an intricate mesh as in the adult spleen, with sinus formation, and ill defined clusters of lymphocytes are present. Complete regeneration of large transplants occurs from the 12th to the 21st day and the morphological structure of adult spleen tissue is apparent, with well developed capsule and trabeculae.

These studies suggest that the reticular cell of the adult spleen retains its potentiality for differentiation and may be the precursor of the structural elements of the spleen.

ADDENDUM: Some months after this work had been completed a report on the behavior of transplanted spleen by Silberberg appeared (*Arch. Path.*, 1935, **20**, 216). In autotransplantation of splenic tissue he also observed complete regeneration in 16 to 21 days. He finds the reticular cells the most resistant to necrosis but does not commit himself with reference to the transformation of these cells to lymphoblastic tissue.

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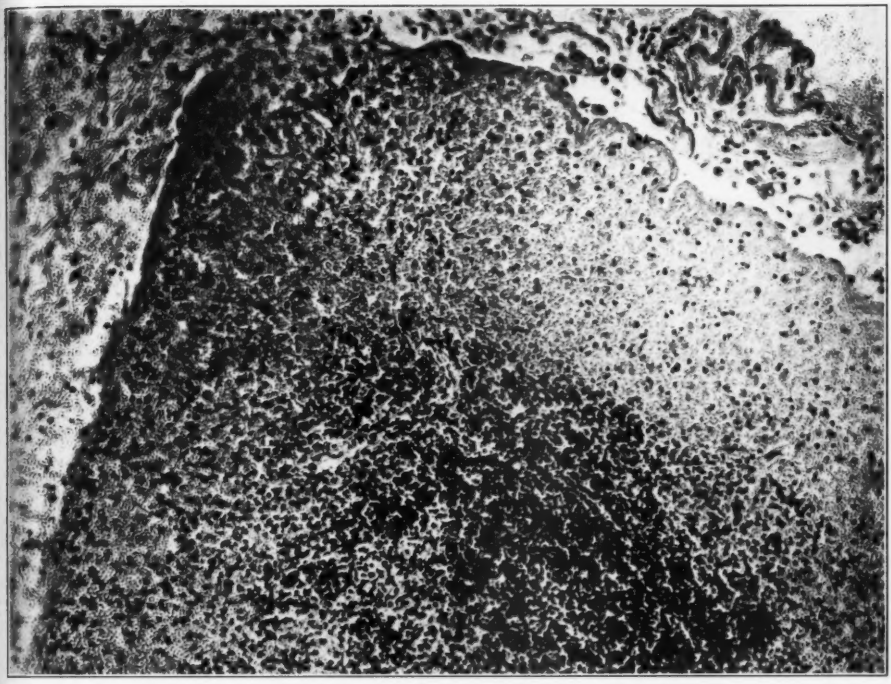
DESCRIPTION OF PLATES

PLATE 121

FIG. 1. Autoplastic splenic transplant, 48 hours. Necrosis of inner portion of the transplant. Outer zone contains numerous reticular cells growing into the transplant and a few scattered lymphocytes. Hematoxylin and eosin stain. × 100.

FIG. 2. Autoplastic splenic transplants, 3 days. Small transplant showing complete replacement by reticular cells and sinuses. Few lymphocytes present. Numerous sinusoidal structures lined with flattened endothelium. In some sinuses endothelial cells are present in the lumens. The reticular cells fill the intersinusoid spaces. Hematoxylin and eosin stain. × 440.



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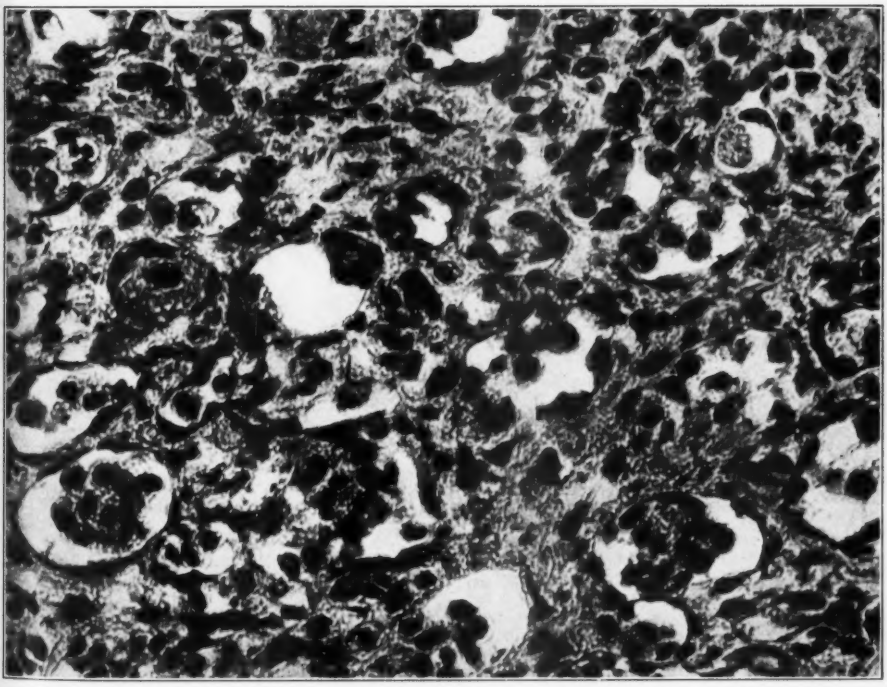

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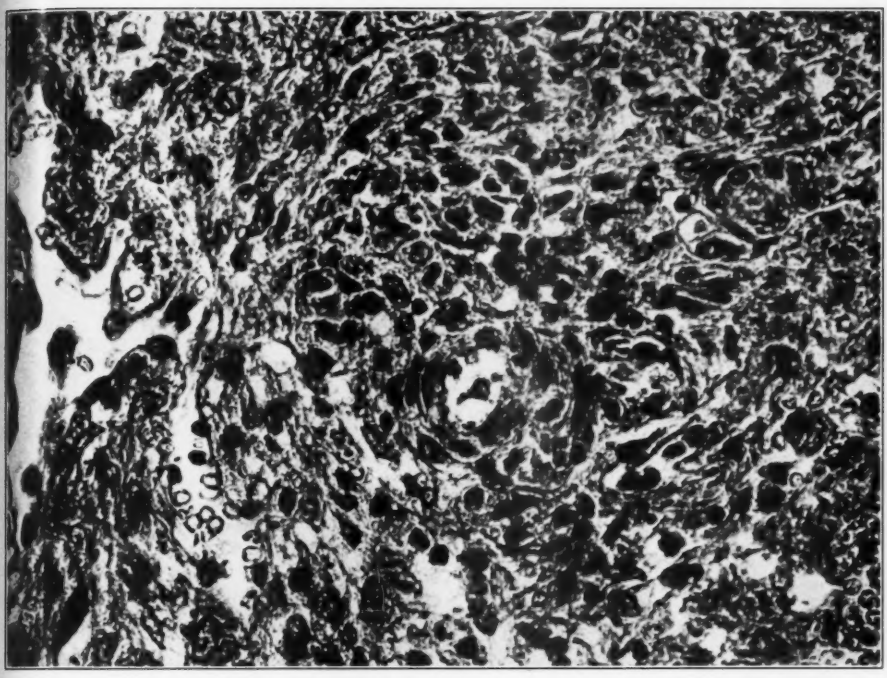
PLATE 122

FIG. 3. Autoplastic splenic transplant, 4 days. Area showing early follicle formation. The reticular cells are arranged in a concentrically lamellated whorl about a capillary. A few lymphocytes are present within this primitive lymph follicle. Hematoxylin and eosin stain. × 440.

FIG. 4. Autoplastic splenic transplant, 7 weeks. Completely regenerated transplant showing adult splenic structure. Note presence of megakaryocytes in the pulp. Hematoxylin and eosin stain. × 240.



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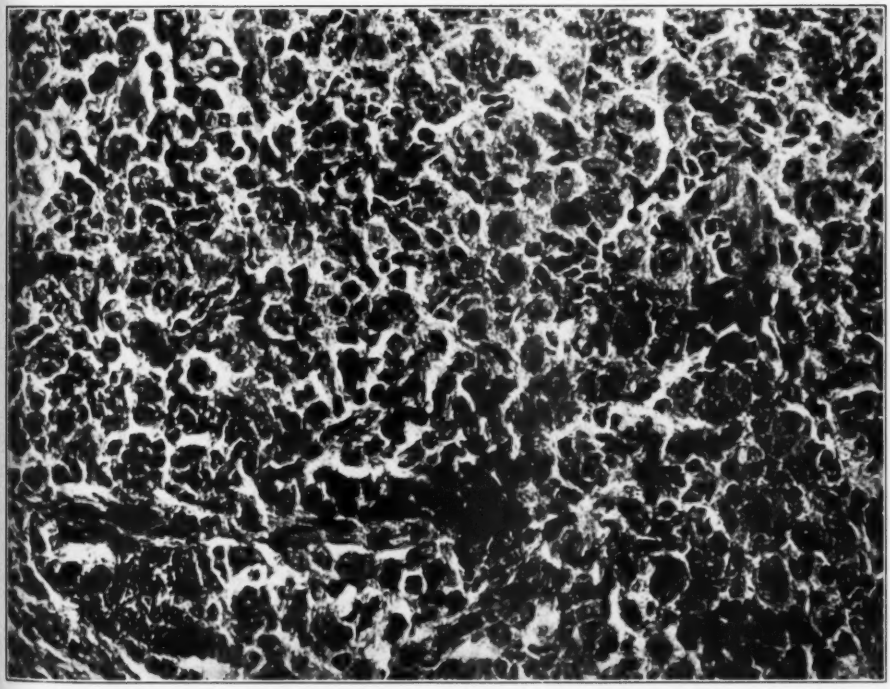

4

Perla Regeneration of Autoplastic Splenic Transplants

MENINGOCOCCUS MYOCARDITIS *

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Myocarditis due to the meningococcus is apparently an extremely rare disease, judging from the paucity of the relevant literature. With the exception of the reports of Westenhoeffer [1] and Gruber,[2] which are quoted in some of the more comprehensive reference books, case reports of meningococcus myocarditis could not be found. There are a number of instances of meningococcus endocarditis reported in the literature. When these case reports were studied, lesions in the myocardium were encountered which the respective authors merely mentioned and which apparently were not considered to be significant. The purpose of this communication is to stress the occurrence and the clinical and pathological significance of meningococcus myocarditis, to list the changes in the myocardium described in the literature, and to report 2 cases of myocarditis in two patients who died of meningococcus meningitis.

REVIEW OF LITERATURE

Klebs [3] in 1865 described an enlarged heart associated with acute endocarditis of the mitral valve in a case of epidemic meningitis. The skeletal muscles revealed an increase in connective tissue cells with a few abscesses and some necrosis. He maintained that the myocardium revealed similar changes which, however, were not described.

Warfield and Walker [4] in 1903 reported a case of acute ulcerative endocarditis caused by meningococci. The heart muscle, grossly, was soft and friable, brownish gray, and streaked with white and yellow lines, but was not described histologically.

Westenhoeffer [1] in 1906 found minute leukocytic infiltrations in the myocardium in 3 cases of epidemic meningitis. He described inter- and intramuscular leukocytic infiltrations, some of which were circumscribed and others diffuse.

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Cecil and Soper [5] in 1911 described a slight increase in connective tissue in the myocardium in a case of meningococcus endocarditis.

Netter and Debré [6] in 1911 stated that heart complications are rare. They maintained, apparently quoting Westenhoeffer, that the myocardium of patients dying from fulminating meningococcus sepsis may show small areas of inflammation caused by meningococci.

Finley and Rhea [7] in 1912 described a "red" myocardium in a case of meningococcus endocarditis, but gave no histological description.

Jochmann,[8] in his textbook on infectious diseases, in 1914 stated that the myocardium often showed intramuscular round cell infiltrations which were partly diffuse and partly circumscribed, and which in some cases might explain death from myocardial failure. However, none of his own observations was given.

Gruber [2] in 1916 stated that of 15 cases of meningococcus meningitis, 8 revealed unquestionable myocarditis, while 7 showed only minute areas of inflammation. The inflammatory lesions could not be interpreted as miliary abscesses. Occasionally the inflammatory cells were perivascular in distribution. In one instance areas of necrosis, fibroblasts and giant cells were encountered, findings that suggested the possibility of a rheumatic infection. None of the sections of the myocardium revealed the presence of microorganisms.

Ghon [9] in 1916 described a case of meningococcus septicemia in which the myocardium showed acute inflammatory changes and many meningococci.

Krumbhaar and Cloud [10] in 1918 reported 3 cases of acute meningococcus endocarditis. A flabby heart was described grossly in only 1 case.

Herrick [11] in 1918 summarized the heart findings in 31 autopsies. A fibrinopurulent pericarditis was found in 4. In 4 others there was a seropurulent pericarditis. Hypertrophy of the heart, dilatation of the heart and acute tricuspid endocarditis were found respectively in 3 other cases. Histological examinations of the myocardium were not reported.

Worster-Drought and Kennedy [12] in 1919 described a large soft heart. The heart was examined only grossly and the diagnosis of myocarditis was made. It was described as follows: "There were mottled, small pale areas in the myocardium alternating with con-

gested areas, and the heart muscle fibers showed a tendency to fall apart."

Kennedy [13] in 1926 described a brown atrophy of the heart in a case of meningococcus septicemia.

Rhoads [14] in 1927 described a vegetative endocarditis due to meningococci. Microscopically a thickened epicardium was infiltrated with lymphocytes. Between the muscle fibers there were many endothelial leukocytes, polymorphonuclear neutrophiles, eosinophiles and lymphocytes. A few areas showed necrotic bundles of collagen fibers surrounded by endothelial leukocytes, strongly suggesting Aschoff bodies. In certain areas there was fairly extensive necrosis of muscle fibers with replacement by granulation tissue. Meningococci were not described in the myocardium.

Moleen and Seecof [15] in 1929 described hemorrhages in the endocardium and pericardium in their case of meningococcus septicemia. The heart was soft and flabby.

MacMahon and Burkhardt [16] in 1929 stated that the myocardium in their case of meningococcus endocarditis showed surprisingly few changes. There were a few, small, perivascular foci of polymorphonuclear leukocytes, and occasional necrotic muscle fibers were invaded by endothelial leukocytes.

Baehr and Klemperer [17] in 1929 found multiple foci of necrosis and lymphocytic infiltrations in the myocardium in their fulminating cases of meningococcus bacteremias. Some of the polymorphonuclear leukocytes contained a few Gram-negative diplococci.

Bancker [18] in 1930 noted eosinophilic cells, lymphocytes, endothelial leukocytes and polymorphonuclear leukocytes between the heart muscle fibers in a case of meningococcus endocarditis. There were also a few areas containing necrotic bundles of collagen fibers which were surrounded by endothelial leukocytes, thus resembling Aschoff bodies. Necrosis of muscle fibers with replacement by granulation tissue was also present. His findings were practically similar to those of Rhoads.

Gwyn [19] in 1931 described myocardial changes in a case of subacute meningococcus endocarditis. A few polymorphonuclear leukocytes were found between the heart muscle fibers and there was edema of the connective tissue. Also, some scarring was observed along the vascular channels which indicated the presence of chronic myocarditis. This moderate perivascular fibrosis was diffuse and

in many instances nodular. The picture was suggestive of a rheumatic infection but no Aschoff bodies were seen.

Stevenson [20] in 1931 reported a case of chronic meningococcus septicemia with bacterial endocarditis. He described minute abscesses in the wall of the right ventricle in which Gram-negative intracellular diplococci could be demonstrated in the pus cells. There was also an early necrosis of the muscle fibers.

Lemierre, Laporte, Reilly and Laplane [21] in 1934 described severe changes in the myocardium in a case of meningococcus endocarditis. The normal muscle fibers were replaced by a homogeneous material. Other fibers were separated by proliferation of connective tissue.

Ghon [22] in 1934 in a case of acute insufficiency of the suprarenals in meningococcus septicemia described polymorphonuclear leukocytes in the interstitial tissue surrounding minute blood vessels of the heart muscle. Many Gram-negative intra- and extracellular diplococci were found in the myocardium.

This survey of the literature reveals the apparent rarity of meningococcus myocarditis. As can be seen, observations of isolated cases of meningococcus myocarditis not accompanied by meningococcus endocarditis were reported only 5 times. It is interesting that in 1 of these cases, as well as in 4 others associated with endocarditis, accumulation of cells with necrosis was found in the myocardium in such an arrangement as to suggest Aschoff nodules. The possibility that myocardial changes may lead to circulatory failure is hardly mentioned.

In the following, 2 cases of meningococcus myocarditis will be reported briefly. In 1 case the disease was fulminating and took a rapid course, the patient having been ill only about 50 hours. In the 2nd case the patient had apparently improved from a meningococcus meningitis, but later developed signs of heart failure and died.

Case Reports

Case 1. An adult white male, 19 years old, was admitted in a comatose condition. The history revealed that he was in good health up to 1 day before admission, but gradually began to feel drowsy and lapsed into coma. He had had momentary dizzy spells for 2 to 3 weeks prior to admission.

Physical examination revealed small hemorrhagic spots all over the body and in the conjunctiva. The neck was rigid. The Kernig reaction was positive bilaterally. The heart was not enlarged but there was an inconstant diastolic blowing murmur at the apex. The temperature was 39° C. The pulse rate was 124

beats per minute and the arterial blood pressure 100/70. The white blood count was 26,100. There were 80 per cent neutrophilic polymorphonuclear leukocytes, 8 per cent lymphocytes, and 12 per cent immature leukocytes. A spinal puncture was performed and 15 cc. of a thick purulent liquid were removed. The cerebrospinal fluid contained 18,500 cells per cmm. Smears revealed Gram-negative intracellular biscuit shaped diplococci which morphologically showed the characteristics of meningococci.

The content of glucose of three successive specimens of cerebrospinal fluid was 0, 10 and 18 mg. per 100 cc. respectively.

The patient became markedly cyanotic and died 30 hours after admission. The clinical diagnosis was meningococcus meningitis. The possibility of the presence of a meningococcus endocarditis was also considered.

Postmortem Examination

Autopsy revealed an acute purulent leptomeningitis; cloudy swelling of the liver and kidneys; passive hyperemia of the liver; an early bronchopneumonia; and petechial hemorrhages of the skin, pericardium and pelves of the kidneys. There was a generalized hyperplasia of the lymph nodes. The heart findings will be given in more detail.

The heart was slightly enlarged, weighing 375 gm. The pericardial cavity contained 150 cc. of seropurulent liquid and the surfaces were dull, finely granular and contained a small amount of fibrin. The mural endocardium was smooth and glistening and there were no thrombi. A few petechial hemorrhages were found throughout the endocardium but were particularly pronounced in the region of the interventricular septum close to the base of the aortic valve. The myocardium on section was grayish red and showed a number of red dots and reddish gray streaks.

Histological Examination

Histological examination of the heart showed an epicardium that was covered with fibrin and enmeshed polymorphonuclear leukocytes. The subepicardial vessels were markedly dilated. The subendocardial portions, particularly in the region of the bundle of His, showed many extravasated red blood corpuscles separating the individual fibers from one another. Scattered throughout the myocardium were a number of polymorphonuclear leukocytes, mainly arranged in groups, which interrupted the course of the heart muscle fibers and also extended into the interstitial tissue. A number of red blood cells and large endothelial leukocytes were also

found in these regions which showed the very recent inflammatory exudate. Occasionally large cells with basophilic granules were observed in the Giemsa preparations. Throughout the sections necrotic heart muscle fibers were recognized, which were separated from one another by polymorphonuclear leukocytes, red blood cells and endothelial leukocytes. Some of the perivascular spaces contained deposits of fibrin and a few polymorphonuclear leukocytes. Outspoken infarcts were not observed, but there were minute and larger areas of necrosis which were infiltrated by polymorphonuclear leukocytes. The Gram-Weigert stain revealed a number of biscuit shaped diplococci. They were, in part, intracellular within the polymorphonuclear leukocytes, and in part extracellular. The Rudnikoff-Stawsky [23] modification of the Gram stain indicated that they were Gram-negative. The muscle fibers throughout showed indistinct striations. The intercalated discs were prominent.

Summary

A 19 year old male who developed a meningococcus meningitis died abruptly after developing a severe cyanosis. The autopsy revealed, in addition to the meningococcus meningitis, an acute myocarditis which undoubtedly was the result of a meningococcus septicemia.

CASE 2.* A 22 year old female was admitted to the hospital complaining of drowsiness, headache, vomiting, fever of 2 days duration and a body rash. She was perfectly well until 3 days before admission.

Physical examination revealed an acutely ill, white female. The temperature was 38.5 C. The pulse beats were 132 per minute with extrasystoles; the arterial blood pressure was 114/85. The neck was rigid and any motion of the head caused intense pain. There was impaired resonance over the right lung. The heart was within normal limits and there was a systolic murmur over the pulmonic area and the upper sternum. The electrocardiogram revealed a sinus tachycardia. Examination of the blood showed 3,920,000 red blood cells. The hemoglobin was 70 per cent. There were 25,900 leukocytes, 95 per cent of which were neutrophilic polymorphonuclear leukocytes, and 5 per cent lymphocytes. The urine contained traces of albumin. A spinal puncture was performed and 30 cc. of cloudy fluid removed. The content of glucose of the cerebrospinal fluid varied from 13 to 46 mg. per 100 cc. The fluid contained 18,000 polymorphonuclear leukocytes, and smears revealed intracellular Gram-negative diplococci which showed the morphological characteristics of meningococci. Meningococci were cultured from the cerebrospinal fluid. The organisms were also identified

* This case is referred to in the chapter on meningitis by Levinson in Practice of Pediatrics by J. Brennemann. W. F. Prior Co., Inc., Hagerstown, Md.

by agglutination with a standard antimeningococcus agglutinating serum on two occasions in dilutions of 1:160 and 1:320 respectively. The blood culture taken on one occasion remained sterile.

The patient was given intraspinal and intravenous injections of antimeningococcus serum. After a temporary improvement, when she was thought to have recovered from the meningitis, she developed subconjunctival ecchymoses, gradually became cyanotic and died 30 days after admission.

Postmortem Examination

Autopsy revealed an acute fibrinopurulent meningitis; acute meningo-encephalitis; thrombosis of the meningeal veins and longitudinal sinus; cloudy swelling of the liver and kidneys; acute splenic hyperplasia; a recent bronchopneumonia; and an interstitial pneumonia. There was also passive hyperemia of the lungs, liver, spleen and kidneys.

The heart was about normal in size and shape, weighing 300 gm. A few mural thrombi were found in the right auricle. The valvular apparatus was intact. The subendocardium of the left ventricle in the region of the interventricular septum showed several petechial hemorrhages. The myocardium throughout was grayish and of a "boiled" appearance.

Histological Examination

Histological examination of the myocardium showed swollen heart muscle fibers with indistinct striations. The subendocardial regions corresponding to the interventricular septum of the left ventricle showed many extravasated red blood corpuscles which were found more or less confined to the bundle of His. Close to the hemorrhagic zones a number of polymorphonuclear leukocytes were present infiltrating the interstitial tissue between the muscle fibers. Only débris of the latter was found in these regions. In other fields the polymorphonuclear leukocytic infiltration was more diffuse, involving both the parenchyma and the interstitial tissue. A number of endothelial leukocytes and many red blood corpuscles were found among the polymorphonuclear leukocytes. There were also present large cells with coarse basophilic granules and rarely a few lymphocytes. In a few regions the inflammatory cells were confined to the perivascular spaces. The Gram-Weigert stain revealed a number of biscuit shaped diplococci, partly intra- and partly extracellular. The Rudnikoff-Stawsky [23] modification of the Gram

stain showed that the organisms were Gram-negative. A culture from the myocardium was not taken. Neither the endocardium nor the pericardium revealed histological changes.

Summary

A 22 year old female was admitted with symptoms of meningitis. She seemed to improve from the meningitis but gradually developed cyanosis and died. At autopsy, in addition to a meningo-encephalitis, an acute myocarditis was found which was the result of the meningococcus infection.

Comment

These 2 cases are characteristic, inasmuch as they clinically revealed evidence of circulatory failure and anatomically a myocarditis. In the 1st case the circulatory collapse occurred rapidly, the patient having been ill only 2 days, but in the 2nd the circulatory failure developed rather slowly. Both patients showed meningococcus meningitis. In the 1st case the culture of the cerebrospinal fluid remained negative. The short stay of the patient in the hospital prevented obtaining another specimen. Smears from the cerebrospinal fluid, however, showed the characteristic Gram-negative intracellular biscuit shaped diplococci. Because of this finding and the presence of the intracellular Gram-negative diplococci in the inflammatory lesions of the myocardium, it seems clear that the cause of the myocarditis was a meningococcus bacteremia, even though absolute proof is missing. In the 2nd case a pure culture of meningococcus was obtained from the cerebrospinal fluid, in addition to finding the organism in the smears from the cerebrospinal fluid and in the sections of the heart muscle.

As stated above, observations on meningococcus myocarditis are exceedingly rare. Relatively more common a type of myocarditis is reported, which occasionally is found associated with meningococcus endocarditis, though this type of endocarditis *per se* is also a rare occurrence. In none of the reported cases, or in these 2 instances, was the myocarditis diagnosed grossly or was it even suspected.

Histologically the myocarditis is characterized by the hemorrhagic exudate, the early appearance of large endothelial leukocytes, the early destruction of muscle fibers and the presence of intracellular Gram-negative diplococci. The foci of necrosis are similar to those

seen in the myocardium in instances of subacute bacterial endocarditis.[24] Outspoken abscesses were missing. In neither of the two hearts were granulomatous lesions seen similar to those described by other authors. Although in both hearts the lesions were more or less diffuse, in each the bundle of His was involved, revealing polymorphonuclear leukocytic infiltrations and hemorrhage. Attempts toward healing were not observed.

Since the 1st case of meningococcus myocarditis reported was observed, special attention has been given to the myocardium of 9 additional cases of meningococcus meningitis. One of these constitutes the 2nd case here reported. In other words, meningococcus myocarditis was found twice among 10 cases of meningococcus meningitis. This fact is in contradiction to the prevailing opinion as to the extreme rarity of meningococcus myocarditis which one is forced to accept after study of the pertinent literature. Meningococcus myocarditis perhaps would be considered less rare if the myocardium of relevant cases was examined more carefully.

In view of the prevailing opinion that the primary lesion in meningococcus meningitis is a meningococcus bacteremia (Herrick[11]), it is not surprising that occasionally meningococci may be the cause of an isolated myocarditis. It is likely that the associated myocarditis with resulting myocardial failure seriously influences the prognosis, not only because of the impending myocardial failure but also because it seems that the myocarditis is the result of an overwhelming infection with meningococci. The abrupt death of the first patient may be explained by the overwhelming infection with meningococci, resulting in myocarditis.

Summary and Conclusions

Observations on isolated meningococcus myocarditis not accompanied by meningococcus endocarditis are rare, as far as one can judge from the relevant literature. However, because of the fact that meningococcus myocarditis was found twice among 10 cases of meningococcus meningitis, which were carefully studied for inflammatory changes in the heart muscle, the disease is apparently not so rare as one would expect from the literature.

Two cases of meningococcus myocarditis are reported. In 1, the patient developed signs of meningitis and died with marked cyano-

sis about 50 hours after the onset of symptoms. The 2nd patient, who also had typical symptoms of meningitis, had apparently improved when cyanosis developed and death occurred unexpectedly.

Histologically meningococcus myocarditis is characterized by a hemorrhagic exudate, the early appearance of endothelial leukocytes, destruction of muscle fibers and the presence of intracellular Gram-negative diplococci. There are also foci of necrosis which, however, are similar to those seen in the myocardium of cases of subacute bacterial endocarditis.

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DESCRIPTION OF PLATE

PLATE 123

FIG. 1. Note the necrotic heart muscle fibers and the hemorrhagic exudate. Iron hematoxylin-eosin preparation. × 350.

FIG. 2. Note the fibrin in the perivascular space. Iron hematoxylin-eosin preparation. × 300.

FIG. 3. Acute myocarditis. Note destruction of heart muscle fibers. Iron hematoxylin-eosin preparation. × 800.

FIG. 4. Note the intracellular cocci. Gram-Weigert stain. × 1800.

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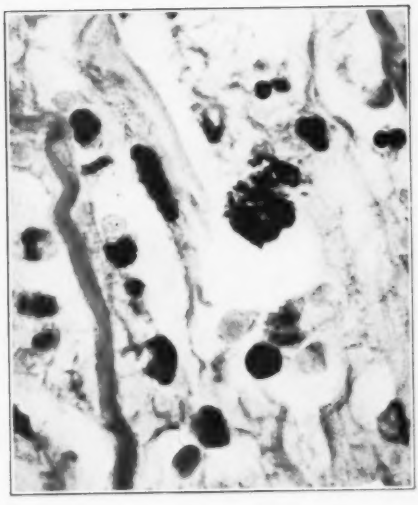

4

Saphir

Meningococcus Myocarditis

THE RELATION OF DIET TO THE OCCURRENCE OF GASTRIC LESIONS IN THE RAT *

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Hyperplastic and ulcerative lesions have been observed in the stomachs of rats which we feel should be thoroughly investigated as to etiology, not only because an explanation of their cause might contribute to the knowledge of proliferative lesions in general, but also because an understanding of the mechanism of their development might disclose distortions in alimentation which accompany the formation of proliferative and ulcerative lesions in the stomach. A brief résumé of previous investigations concerning these lesions will indicate the confusion that exists regarding their etiology. They were first described in 1913 by a British investigator, Singer,[1] who believed them infectious in origin. He reported their presence in the stomachs of rats because Fibiger [2] was at that time working on the relation of Spiroptera to gastric carcinoma in the rat and similar lesions were described as precarcinomatous. It was not until 1923, however, that Pappenheimer and Larimore [3] demonstrated for the first time that the lesions were in some way related to a deficiency in the diet. They did not discover the exact nature of the deficiency but they did find that neither cod liver oil, nor Osborne and Wakeman's yeast extract, nor a change in the mineral content protected against these lesions.[4] Pappenheimer and Larimore felt, however, that the ingestion of hair was a contributory factor. On the other hand, Fujimaki, Kimura, Wada and Shimada [5] (1927) believed that the lesions were caused by a deficiency of vitamin A, and in this conclusion Santi [6] and Bisceglie [7] have agreed. If, however, a deficiency of vitamin A were the etiological factor one would expect that Wolbach and Howe,† [8] who carefully studied the pathological

* Received for publication February 20, 1936.

† In the experiments of Wolbach and Howe the animals were forced to eat all the rations each day in spite of loss of appetite from the vitamin deficiency. The animals did not suffer from other food deprivations therefore. Such precautions were not reported by the other investigators studying vitamin A deficiencies.

changes accompanying A avitaminosis, would have noted them, but instead they found only atrophy of the mucosa of the stomach.

A new suggestion as to the etiology of the lesions was recently proposed by Hoelzel and DaCosta,[9] and Carlson.[10] They suggested that the low protein content of the diet was responsible, for the lesions did not develop when large amounts of casein were given. The adequacy of vitamin control in their experiments, however, was not mentioned. Alternate days of fasting, they found, increased the number and severity of the lesions, and the lesions occurred in the glandular portion of the stomach as well when the deficiency was well advanced.[10] Steel filings incorporated in the diet as an irritant in place of hair did not produce the lesions when the diet was adequate, but when it was inadequate irritation made the lesions more extensive.

Büchner, Siebert and Molloy [11] produced the lesions in rats by increasing their gastric secretion with injected histamine. The character of diet given to these animals was not mentioned, but it was said that alternate days of fasting increased the incidence of the lesions, that hair in the diet did not produce them, and that when the rats ate feces no lesions resulted in spite of the injections of histamine. Lastly, Findlay [12] has described papillomatous proliferation in the squamous epithelium of the rumen portion of the stomach with B_2 deficiency but not with a B_1 deficiency.

From this brief review of the literature it should be apparent that the exact nature of the deficiency of the diet which produces the lesions has not been solved and that the problem is worthy of further investigation because of the possible relation of diet to tissue hyperplasia.

Experimental

The general plan of our investigations was to produce the lesions with a deficient diet in a definite length of time and then to add to it various factors in order to discover which ones would provide protection. The influence of the age of the animal on the production of the lesions in relation to the diet was controlled by studying the occurrence of the lesions in both young and adult rats fed on the different diets. The young rats used were 6 weeks old, while the adults were over 4 months of age. Both albino and hooded rats were employed in order to eliminate the possibility of a strain of

rats particularly susceptible to the development of the lesions. They were kept in separate wire-bottom cages and 10 were fed each diet. The entire data for the experiments, including the weights of the animals taken at the beginning of the experiment and when killed, are given in Table I. At autopsy the stomachs were removed, distended with formalin and allowed to fix before they were examined. This technic facilitated the study of the normal microscopic anatomy of the stomachs and the pathological changes therein.

For controls, 75 rats were fed an adequate stock diet.* The stomachs of all these animals were without lesions. Microscopic sections were made of the various portions of these stomachs in order to furnish a picture of the normal microscopic anatomy.

The lesions were then produced by feeding rats a modified Pappenheimer and Larimore diet consisting of flour and salt mixture (Diet 84).[4] After 42 days on this regimen 9 out of 10 young rats developed lesions in the stomach. At the beginning of the experiment they ate about 6 to 8 gm. of food daily, but as they continued on the diet their appetites fell off gradually until finally they consumed only about 4 to 5 gm. daily. Most of them developed xerophthalmia and failed to gain weight (Table I). Only two of the stomachs examined contained hair. Most of the lesions were in the rumen, but five were in the glandular portion of the stomach as well (Figs. 1 and 2).

While in this length of time Diet 84 readily produced the lesions in the stomachs of young rats, it did not do so in the stomachs of the adult rats. In fact, 10 adult rats fed Diet 84 daily for 61 days had no ulcers, but when the adult rats were starved every other day and fed Diet 84 on the alternate days, then 7 out of 10 developed lesions in the stomach in 49 days. Likewise, when the food intake of the adult rats was restricted to 5 gm. daily, 6 out of 10 developed lesions in 42 days. However, when the complete diet was fed to control rats in such a manner that the animals lost a corresponding amount of weight over the same length of time, then the lesions did not develop. It is obvious from these experiments that young rats are more sensitive to the deficiency of the diet than are adult rats and that weight loss alone does not account for the formation of the lesions.

* Calf meal diet.

TABLE I

Experiment No.	No. of rats	How fed	Days on diet	Age	Diet	Weight changes	Lesions
Controls	50	Daily	50	Adult	Normal Zucker	+8%	0
"	25	Daily	Life span	Young	calf meal	..	0
					Lesion-Producing Diet — Diet 84		
31	10	Daily	42	Young	84	−5%	9
37	10	Daily	61	Adult	84	−8%	0
11	10	Alt. days	49	Adult	84	−41%	7
Y1	10	Daily. Restricted 5 gm.	42	Adult	84	−35%	6
					Diets with Vitamins A–D		
2	10	Daily	42	Young	84 + 5% CLO*	+14 gm.	5
20	10	Alt. days	50	Adult	84 + butter	..	4
21	10	Alt. days	50	Adult	84 + carotin	..	5
Y4	10	Alt. days	42	Adult	84 + 5% CLO	−22%	6
24	10	Daily	52	Young	Moise + CLO	+47.6 gm.	1
					Diets with Yeast		
3	10	Daily	42	Young	84 + 5% yeast	+1%	0
Y8	10	Alt. days	42	Adult	84 + 5% yeast	−16%	1
Y9	10	Daily	42	Young	84 + 5% yeast	+72%	0
22	10	Alt. days	50	Adult	84 + 5% yeast	−12.2 gm.	3
29	10	Alt. days	52	Adult	84 + 10% yeast	−31 gm.	0
4	10	Daily	50	Young	84 + 5% CLO + 5% yeast	+2.3 gm.	0
18	15	Daily	44	Young	84 + 5% yeast + hair	..	1
Y2	9	Alt. days — restricted	42	Adult	84 + 5% yeast	−13%	4
					Miscellaneous		
5	10	Daily	42	Young	84 + 20% casein	+11.3 gm.	3
6	8	Alt. starvation	40	Adult	84 + yeast + 20% casein + histamine	+2.6 gm.	0
Y113	10	Alt. starvation	42	Adult	84 + histamine	−28%	5
Y16	10	Alt. starvation	42	Adult	84 + feces	..	0

* CLO = cod liver oil.

Pathology of the Lesions

In the rumen portion of the stomach the smaller lesions were, grossly, round ulcerated areas with heaped up, light yellow circumferences. The edges were not undermined. The more extensive ones were more irregular in shape and tended to have one longer axis. The blood vessels, most prominent on the serosal surface, were more numerous around the lesions than elsewhere and radiated in a stellate pattern from the defects. Food particles frequently adhered to the ulcerated surfaces. Between the ulcers yellowish plaques of tissue of firmer consistence were found and these were sometimes so numerous that they thickened the entire rumen portion of the stomach. The ridge between the squamous and glandular portions of the stomach was frequently hypertrophied (Figs. 1 and 2).

Microscopically the ulcerations occurred in hypertrophied squamous epithelium; that at the margins and that of the plaques was hyperplastic, thickened and often reduplicated on itself. There was exfoliation of the keratin layer (Figs. 3 and 4). The processes of squamous epithelium extending downward toward the muscularis mucosa were widened and frequently caused thinning of this structure. Instances have been reported where the continuity of the muscularis mucosa has been broken by these downward extensions.[5] The subjacent connective tissue both above and below the muscularis mucosa was edematous and in some areas infiltrated with leukocytes, especially near the ulcerations. Mitotic figures in the epithelium were numerous, but the cells were not anaplastic. Superficial necrosis, not extensive enough to cause ulceration, had also occurred on the surface of some of the hyperplastic plaques. The surfaces of the ulcers were, microscopically, covered with the débris of keratin, dead epithelial cells and leukocytic fragments. There was no regenerating fibrous tissue seen in the bases of the ulcers nor was there regeneration of epithelium at the edges of the defects. The ulcerations had, therefore, occurred recently.

In the glandular portion of the stomach the incidence of the lesions was greatest in the pyloric antrum, but they were found over the entire secreting area (Fig. 2). In general, they were smaller and more punched out than those in the rumen, but their edges were also heaped up. Microscopically the individual glands of the epithe-

lium were elongated, their ends blunted and separated. The ulcerations occurred in hyperplastic areas and consisted of craters with steep walls (Fig. 5). Cellular infiltration adjacent to the ulcers was common. The non-hyperplastic areas of epithelium showed no evidence of gastritis similar to that seen in the gastric mucosa of the human stomach with peptic ulcer.

It is apparent, therefore, that the pathological changes were the same in both the rumen and the glandular portions of the stomach, consisting essentially of areas of hyperplasia with ulceration.

Relation of Vitamins A and D to the Development of the Lesions

To Diet 84 adequate amounts of vitamins A and D were supplied by the addition of 5 per cent cod liver oil. The young rats fed on this mixture for 42 days ate and grew better than those on Diet 84 alone, but 5 out of 10 of them developed lesions in the stomach. Likewise, 6 out of 10 adult rats starved every other day and forced to take cod liver oil on the alternate days developed the lesions. Carotin and butter, employed as sources of vitamin A, also failed to provide protection against the lesions even though the rats were forced to eat these substances separately. It was therefore concluded that vitamin A did not protect against the lesions.

Effect of Whole Yeast

Five per cent whole yeast was then added to Diet 84 to supply the B complex. The 10 young rats fed on this mixture were without lesions in the stomach, but 1 of the adult rats starved every other day and fed Diet 84 plus 5 per cent whole yeast on the alternate days had a lesion. The experiment was, therefore, repeated with 10 other adult rats, and 3 developed lesions. For a second trial, young rats were given a different brand of whole yeast (5 per cent), but again no lesions developed. Next, adult rats, starved every other day, were given 10 per cent whole yeast and then absolute protection followed. Moreover, 5 per cent whole yeast plus 5 per cent cod liver oil added to Diet 84 gave absolute protection, and even when the bulk of Diet 84 was increased 25 per cent by the addition of hair, only one lesion developed when 5 per cent whole yeast was given to young rats.

Lastly, when adult rats were fed a sufficient amount of whole

yeast, lesions did not develop although histamine had been injected according to the technic of Büchner. The Osborne and Wakeman yeast extract added to Diet 84, however, did not give protection as Pappenheimer and Larimore had likewise found (Table I). We were, therefore, forced to conclude that whole yeast when given in sufficient amounts can prevent the development of these gastric lesions.

That this protection by whole yeast is not absolute, however, was not only brought out by the above experiments but also by the fact that when the amount of flour and salt mixture was restricted, 5 per cent whole yeast afforded less protection than when a liberal amount of Diet 84 was allowed. Thus, with a restricted amount of Diet 84 plus 5 per cent whole yeast, 4 out of 9 adult rats developed lesions while only 3 of them had lesions when a liberal amount of Diet 84 was allowed. And again, when the diet was made more complete with the addition of other food factors, then the lesions were less frequent although the amount of yeast was kept constant. Thus only 1 young rat developed a lesion after 52 days when cod liver oil and Crisco were added to Diet 84 plus 5 per cent yeast. These facts indicate that the more complete diet has a sparing action on the necessary food element.

Effect of Increased Amount of Protein in the Diet

Finally, because of the work of Hoelzel and DaCosta,[9] 20 per cent vitamin-free casein was added to Diet 84 and fed to young rats daily for 42 days but, when killed, 3 of these animals had lesions in the stomach. On the other hand, with 5 per cent whole yeast added to this mixture no lesions developed.

Discussion

The experiments indicate that whole yeast added to a deficient diet protects against the development of hyperplastic and ulcerative lesions in the stomach of the rat; that young rats are more sensitive to the deficiency of the diet than adults; and that other food factors contributing to the adequacy of the diet have a sparing effect. It follows, accordingly, that when the diet is adequate a longer time is required to produce the lesions with the necessary substance absent than when the diet is less adequate. The amount of neces-

sary substance required to give absolute protection under any circumstance will, in the last analysis, therefore, depend on the age of the animal and the severity of the depletion produced by the experiment and this, in turn, on the manner of feeding and the general adequacy of the food given. The dietary regimen preceding the initiation of the depletion experiment is, of course, a determining factor.

The exact nature of the protective substance in yeast must further be investigated, but the action of one of the contained vitamins is immediately suggested. Carlson [10] has advanced the theory that the lesions were caused by gastric stasis, but such a fact, if true, would only support the contention that the lesions were produced by a vitamin deficiency. The withdrawal of vitamin B from a diet decreases gastric motility while the addition of an adequate amount of the vitamin to a deficient diet causes the motility to return to normal.[13] The greater sensitivity of young rats to an inadequate supply of the necessary substance is, of course, compatible with the picture of a vitamin deficiency, and there is the possibility that other foods might contain traces of the necessary vitamin. Thus, if casein were not specially treated so as to destroy the vitamin content then it is conceivable that the addition of large amounts to a diet would afford protection. This might be the explanation of the discrepancy between this work and that done by Hoelzel and Da Costa.[9] On the other hand, the sparing action of cod liver oil is difficult to explain on a vitamin basis, especially when the oil itself, as shown by Pappenheimer and Larimore, has the same effect after the vitamin content is destroyed.

Actually the discrepancies between our experimental results and those obtained by the previous investigators are not difficult to explain nor are they as great as would be imagined. Some differences have naturally arisen from the failure to control the influence of the age factor and further from the assumption that the mere presence of a food in a diet necessarily guarantees that the desired nutritional benefit will be obtained. For benefit or protection, as the case happens to be here, the necessary substance must be consumed and in adequate amounts, as our investigations have shown. Thus, with the loss of appetite which accompanies prolonged A avitaminosis, there would be an insufficient ingestion of the necessary substance even though yeast were present in the diet, and the lesions

should develop.[5] The Osborne and Wakeman yeast extract previously used by Pappenheimer and Larimore did not contain the necessary factor,[4] so it is quite understandable why these investigators did not suspect the presence of a necessary substance in whole yeast. Findlay, though, in confirmation has described the formation of proliferative lesions in the stomachs of rats fed on a vitamin B-free diet and further demonstrated that the giving of B_1 did not prevent their development.

Pappenheimer and Larimore, and Carlson, have demonstrated that the incidence of the lesions is heightened by the presence of local irritation, and histamine with its resulting secretion of free acid into the empty stomach does irritate the gastric mucosa as Büchner has shown.[11] In Büchner's experiments, therefore, there is the possibility that the repeated flow of juice simply precipitated the lesions in animals previously inadequately fed, or, at least, we were unable to produce the lesions with histamine injections after the animals received sufficient quantities of yeast. Moreover, the sporadic occurrence of the lesions in laboratory rats illustrates the fact that animals can unknowingly be fed in such a way that they are on the borderline of or actually in the deficiency which produces the lesions. Henning and Norpoth,[14] for example, found so many of the lesions in their laboratory rats that they believed Büchner was describing a common disease in the stomach of the rat. Yet, on the other hand, so many well fed animals have been examined particularly for the lesions by Carlson, ourselves and others (Fibiger examined over 2000 wild rats) that one must conclude that Henning's rats were likewise inadequately fed. His finding of the lesions in what he considered normal rats, however, demonstrates how easy it is to assume that an irritative agent produces the lesions when actually the previous diet of the animal is inadequate. Even Büchner's own data support the thesis that his animals were near the deficiency, for starvation aided the production of the lesions, and some of the rats starved and receiving no histamine likewise developed the lesions. Lastly, Büchner believed that feces protected against the lesions because they neutralized the gastric juice secreted after the histamine was injected, but we have obtained the same protection by adding 10 per cent dried feces to Diet 84. Under these circumstances the lesions were not produced by the inadequate diet

because of the presence of feces and yet there were no obvious distortions in the secretion of gastric juice.* The reports, therefore, that feces may contain vitamin B suggest that the action of a necessary food element may again be the answer.

Dalldorf and Kellog,[15] and Büchner, have argued that peptic ulcer in man arises from conditions similar to those experimentally needed to produce the lesions in the stomach of the rat, while others have advanced the hypothesis that the mechanisms involved are similar to those necessary to produce the gastritis which accompanies peptic ulcer. The latter might be a possibility, but certainly there is no similarity between these lesions and peptic ulcer in man. These lesions are acute, are multiple, and the ulcerations occur not only in areas where the epithelium is hyperplastic but in squamous epithelium as well. It is of interest, however, that patients with pellagra have somewhat similar lesions in the squamous epithelium of the lower end of the esophagus and that experimentally hyperplasia can be produced by a dietary deficiency.

Summary and Conclusions

1. Hyperplasia, hyperkeratinization and ulceration of the squamous epithelium of the rumen of the rat's stomach result from an inadequacy in the diet.

2. Similar lesions without keratinization occur in the glandular epithelium as well. The importance of these lesions lies in the relation of diet insufficiencies to tissue hyperplasia.

3. Young rats are more sensitive to the inadequacy of the diet than are adult rats. Adult rats on the same diet must be starved every other day in order to develop the lesions in the same length of time.

4. An adequate intake of whole yeast prevents the formation of the lesions in both young and adult rats. Additional amounts of vitamin-free casein, or cod liver oil, or other forms of vitamin A do not prevent the formation of the lesions. Ingestion of feces prevents their occurrence.

5. The relation of these lesions to peptic ulcer and to previous investigations of their etiology is discussed.

* Bürkle-de la Camp (*Deutsche Ztschr. f. Chir.*, 1929, **220**, 31) has repeated Büchner's investigation and obtained the same result, so the rôle of the gastric juice must be considered important in spite of the above criticism.

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DESCRIPTION OF PLATES

PLATE 124

FIG. 1. Extensive lesions of the rumen. Almost the entire area is hyperplastic, but several ulcerations can be seen.

FIG. 2. Lesions in the glandular portion of the stomach. Note that the entire rumen has the normal smooth appearance (contrast with Fig. 1) except that there is a small lesion beginning in the lower right hand corner. The hypertrophy of the juncture between the two portions of the stomach is clearly shown.

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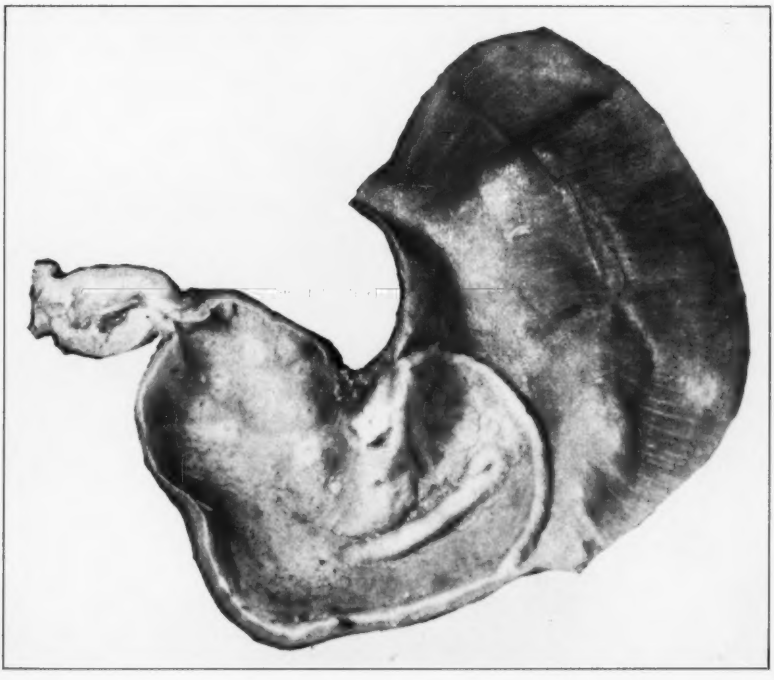

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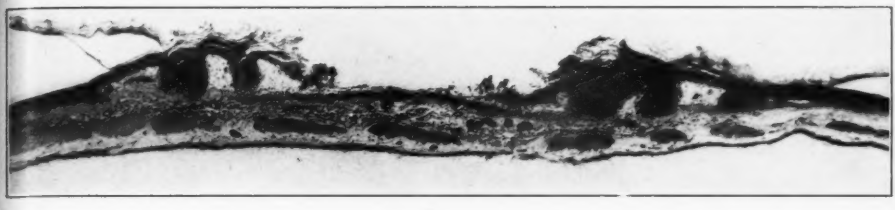
PLATE 125

FIG. 3. Low power magnification of an ulcerated area of the rumen surrounded by hyperplastic squamous epithelium. The normal thickness of epithelium and the normal architecture of tissue layers can be seen at the edges of the section.

FIG. 4. High power magnification of the squamous epithelium at the edge of an ulceration in the rumen. Hyperkeratosis, the increase in thickness and the reduplication of the epithelium into blunt papillary processes pressing on the muscularis mucosa are evident. Edema of the connective tissue between the papillary processes is also apparent. Necrosis of the epithelium at the edge of the ulceration is seen on the right.

FIG. 5. Ulceration in the glandular portion. The ulceration has not extended to the muscularis mucosa and beneath it a collection of lymphocytes is seen. The normal architecture of the epithelium can be seen on either side of the ulceration.



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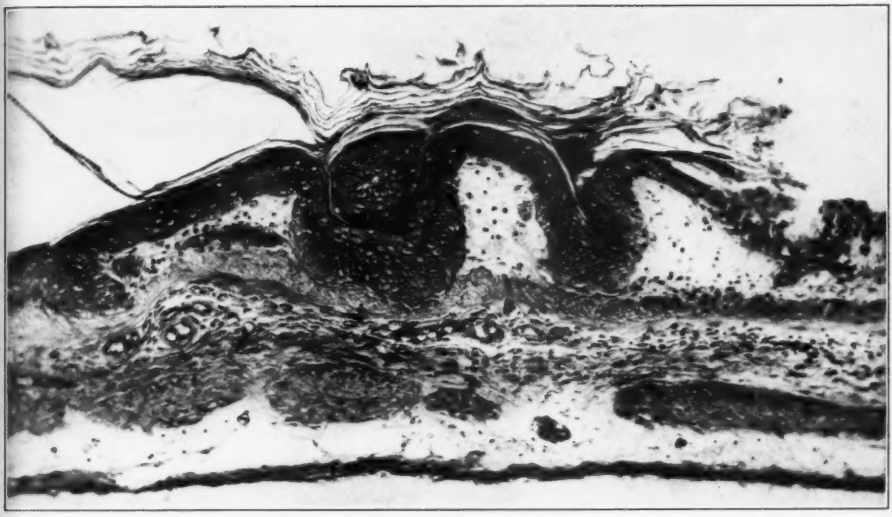

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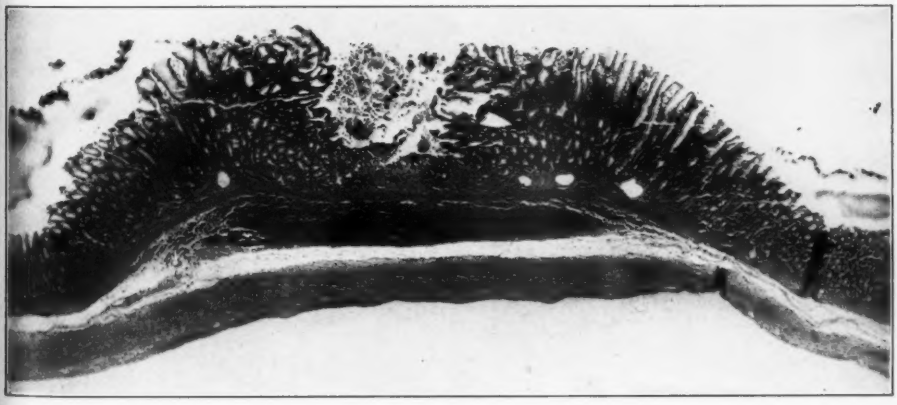

5

AMYLOIDOSIS OF LUNGS AND HEART *

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The subject of amyloidosis of the lungs has received relatively little attention in the medical literature. Virchow [1] in 1857 was apparently the first observer to mention the condition. He described generalized amyloidosis occurring in an aged female suffering from chronic Bright's disease. Small amounts of hyaline substance were present in the small blood vessels and the alveolar walls of the lungs. Iodine followed by sulphuric acid gave reactions typical of amyloid.

In 1909 Schilder [2] presented the findings from 2 cases of chronic pulmonary tuberculosis in which there were widespread amyloid deposits. There was moderate involvement of the blood vessels and alveolar walls of the lungs.

Tsunoda [3] in 1910 stated that of 7 cases of general amyloidosis investigated only 3 had slight amounts of amyloid in the pulmonary vessels and alveolar septa.

The first general discussion of amyloidosis of the lung was made by Kschischo [4] in 1912. He reviewed the cases reported up to that time and added a case of his own, in which there was a considerable amount of amyloid in the spleen and kidneys, and a slight amount in the liver. In the lungs deposits were found in the alveolar septa and in the medium sized vessels. A chronic inflammatory process believed to be syphilitic was present also in the lungs.

Glaus [5] in 1917 described a case of multiple myeloma. There was amyloidosis of the whole intestinal tract and deposits of the substance in the mesenteric vessels, heart, striated muscle, and myeloid tumor of the bone marrow. In places the pulmonary alveolar walls were thickened, in part because of circumscribed, and in part on account of diffuse masses of amyloid.

Lubarsch and Plenge [6] in 1931 stated that 80 cases of amyloidosis at the Berlin University Pathological Institute and 24 cases at the Hindenburg Hospital at Zehlendorf were studied with special care

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to determine the frequency of amyloid involvement of the lungs. Of the total of 104 cases, only 9 presented any evidence of amyloid in the lungs, and in only 1 of these, a patient with chronic pulmonary tuberculosis, was it at all extensive.

In most of these cases there was a concomitant or preceding chronic disease as an etiological factor for the amyloid and the involvement of the lungs was usually part of a general amyloidosis. A brief description of a case which may be considered as a somewhat atypical example of this form of the disease follows. Localized amyloidosis of one lung and fairly extensive deposits of amyloid in the spleen were present. An extensive carcinoma of the bladder and a localized bronchiolectasis of the affected lung were possible etiological factors.

Report of Case

Case 1. *Clinical History:* H. W., a white male, 78 years of age, entered the department of urology (James Buchanan Brady Foundation) of the New York Hospital Nov. 11, 1935. He had had many of the diseases of childhood but was otherwise well until about 4 years before admission to the hospital when a prostatectomy was performed. In June, 1935, he entered the hospital because of urgency, frequency, nocturia and dysuria of 1 years duration. A tumor 5 cm. in diameter, in the right side of the bladder, was removed by means of high frequency current. The tumor did not appear to invade the bladder wall. Radon seeds were placed in the base of the growth. In September, 1935, the suprapubic wound opened, but soon healed following bladder irrigations. Again, in November, 1935, there was a urinary fistula from which came a very foul urine. The bladder was irrigated continuously but the wound did not heal. The patient's blood urea nitrogen rose and 1 day before death there was no excretion of urine. He died Dec. 12, 1935.

Postmortem Examination

Autopsy (No. 8346) was performed 14 hours postmortem. The body was well developed and weighed 48 kg., although there was evidence of loss of weight. The appendix was bound down by fibrous adhesions, and other short fibrous bands extended between several adjacent loops of intestine. The left pleural cavity was obliterated by adhesions, particularly dense at the apex of the lung.

Heart: The heart weighed 370 gm. In the lower part of the left ventricle the wall was decreased in thickness and there was an increase of fibrous tissue. The remainder of the myocardium was not unusual in appearance. The wall of the anterior descending branch of the left coronary artery was thickened in one area and contained

calcium. In the lumen was a small thrombus. In the intima of other branches of the coronary arteries were small atheromatous plaques. The aorta contained a number of these plaques, some of which were calcified.

Lungs: The lungs together weighed 960 gm. The left lung was crepitant throughout and its cut surface was pink and relatively dry. The right lung was similar to the left in appearance, except for an area measuring approximately 5 by 3.5 by 2.5 cm. in the inferior and anterior part of the lower lobe. On section it was found to be somewhat wedge shaped, extending to the diaphragmatic margin, and was composed of firm grayish tissue, near the center of which were several irregular spaces. The largest of these measured about 1.5 cm. in greatest dimension, and several contained thick, yellowish white, purulent material. The cavities were lined by mucosa and communicated with dilated branches of the bronchi. At the apex of each lung was a fibrous scar of the pleura. In the left lung strands of fibrous tissue extended about 1.5 cm. below the scar into the pulmonary substance. A small calcified nodule was found in the lower lobe of the right lung just beneath the pleura of the interlobar fissure. There was a small calcified nodule in a deeply pigmented lymph node situated just above the right bronchus.

Spleen: The spleen weighed 130 gm. The surface was smooth and reddish purple. It was fairly firm and held its shape well on section. Small pale foci, apparently malpighian bodies, were fairly prominent in the light red or pink pulp. The trabeculae were not easily identified. When tincture of iodine was applied to the cut surface the pale foci became brown. These turned to greenish blue when concentrated sulphuric acid was added.

Bladder: The bladder wall was firm and in places measured 2.5 cm. in thickness. Most of the mucosa was replaced by soft, friable, opaque yellow tissue which invaded the wall. The ureteral orifices were not recognized. There were no metastases in adjacent lymph nodes.

Microscopic Examination

Heart: There was a great increase of fibrous tissue in the lower part of the left ventricle and the walls of several small arteries were thickened by fibrous tissue. The lumen of one branch of the anterior descending branch of the left coronary artery was occluded by an

organized but recanalized thrombus, while in the other branch was a fresh thrombus.

Lungs: In general the alveoli were dilated. Many contained scattered polymorphonuclear leukocytes and a few large mononuclear cells. Leukocytes were also found in the walls of bronchioles. In the congested area of the right lower lobe masses of leukocytes were present in the lumens of dilated bronchioles and many lymphocytes and plasma cells were seen in the walls. Fibrous tissue was increased throughout this part of the lung. In addition there was a lighter staining hyaline material present in the walls of blood vessels and forming irregular masses or nodules in the alveolar walls. In areas the lumens of the alveoli were almost completely obliterated on account of the extensive deposits. Many multinucleated giant cells were seen at the margins of the amyloid masses, in places situated in indentations of the substance. In sections prepared by a combined Weigert's elastic tissue and Van Gieson's connective tissue stain the elastic fibers in the blood vessel and alveolar walls were distorted and in places had disappeared. The amyloid was stained dull yellow, in contrast to the bright red of the collagen. Gentian violet and iodine stains were unsatisfactory.

Spleen: Practically all of the lymphoid tissue of most of the malpighian corpuscles was replaced by a pink staining, homogeneous hyaline material, which was also present in places in the adjacent pulp. The walls of the arteries were thickened. Lymphocytes in the pulp were moderate in number. There were numerous plasma cells and scattered large mononuclear cells containing brown pigment granules. Gentian violet stain of a section of alcohol-fixed material gave a metachromatic stain for the amyloid and connective tissue.

Bladder: The innermost parts of the bladder wall were so necrotic that no cells were recognizable. More peripherally there were large masses of epithelial cells separated by delicate trabeculae of connective tissue. In other places the fibrous tissue was denser and more abundant and the cells formed small groups or strands. The epithelial cells varied in size, shape and staining properties with moderately hyperchromatic nuclei. A few multinucleated cells were seen. Mitoses were numerous. The tumor extended through the whole bladder wall.

The principal pathological changes in other organs were metas-

tases from the carcinoma of the bladder to the right adrenal; hydro-ureter, bilateral; hydronephrosis and chronic pyelonephritis, bilateral.

Comment

Somewhat similar to this case are several reported instances of localized deposits of amyloid in the lungs. There was, however, no involvement of other organs. Herxheimer[7] in 1903 found large tumor-like deposits of amyloid in the lung of a male suffering from empyema.

Other examples of localized nodular amyloidosis of the lungs were described by von Werdt,[8] Meyer,[9] and Hallermann.[10] In von Werdt's case there was no obvious etiology. Meyer found generalized arteriosclerosis with atrophy of the kidneys, while in 1 of Hallermann's cases there was empyema and in the other pneumonia.

Atypical Amyloidosis

More frequently amyloid has been observed in the lungs in what has been called atypical or primary amyloidosis. This lesion is characterized, according to Lubarsch,[11] by the frequent absence of an associated disease ("Grundkrankheit"); the occasional nodularity of the deposits; the frequent failure of the deposits to react typically to the specific tests for amyloid; the almost complete absence of involvement of the usual organs; and, on the contrary, the unusual sites of deposit of the amyloid.

The following case of amyloidosis is atypical in a number of respects, and is especially unusual because of the remarkable quantity of amyloid in the lungs and heart.

Report of Case

Case 2. *Clinical History:* F. V., a white male, 52 years of age, was admitted to the medical service of the New York Hospital March 21, 1935. There was no history of significant disease in the family. A number of years before, he was treated for gonorrhea, complicated by urethral stricture. His general health since then had been good, however, until about 1 year before admission when he noted dyspnea on exertion and became more easily fatigued. These symptoms increased in severity during the next 9 months, and 3 months before admission he commenced to have night sweats and noticed some swelling and pulsation of the liver. There was also a chronic cough with production of a large amount of mucus. He was treated with digitalis and curtailed his activities,

but there was little improvement and 3 weeks before admission edema of the lower extremities appeared.

Physical Examination: The patient was well developed and nourished, but was moderately dyspneic. The percussion note was flat over the base of the right lung and breath sounds were absent in that location, as well as in the right axilla. The remainder of the lower lobe and the middle lobe were filled with moist râles. Similar râles were heard in the left lower lobe. The left border of the heart extended almost to the left anterior axillary line. The heart sounds were regular (70 per minute) and of tic-tac quality. A faint, soft systolic murmur was heard over the precordium. The systolic blood pressure was 125 and the diastolic 75 mm. Hg. The abdomen was slightly distended with the suggestion of a fluid wave. The liver edge was palpable a hand's breadth below the costal margin and was smooth and firm. There was slight swelling of the ankles.

Course of Illness: The urine contained 1+ to 2+ albumin and the specific gravity was 1.015 and 1.016 on two examinations. On one examination there were occasional white blood cells. The Kline exclusion and diagnostic tests were negative. The red and white blood cell counts were within normal limits. Four days after admission the blood urea nitrogen was 12 and the blood sugar 118 mg. X-rays of the chest showed an obscuring of both leaves of the diaphragm and the right costophrenic angle. The markings of the visible lung fields were accentuated and in places there was a punctate appearance. The heart shadow was definitely enlarged to the right and left, and the lower trachea displaced to the right.

The patient was given salyrgan intravenously and placed on a maintenance dose of digitalis. The dyspnea remained pronounced and from time to time a moderate amount of blood-tinged sputum was noted. Signs of consolidation of the lungs progressed and the pulse rate rose, varying between 71 and 120. The temperature ranged between 37° C. and 38.8° C., with general upward rise.

Death occurred 14 days after admission.

Postmortem Examination

Autopsy (No. 8078) was performed 11 hours postmortem. The body was well developed and nourished and weighed 47 kg. Several teeth were carious. There were 300 cc. of clear yellow fluid with a specific gravity of 1.015 in the peritoneal cavity. In each pleural cavity were 600 cc. of slightly cloudy yellow fluid with a specific gravity of 1.010.

Heart: The heart weighed 650 gm. In both auricular appendages were masses of friable thrombus, lightly adherent to the endocardium. The valve measurements were normal. The myocardium was firm in consistence with a homogeneous, glassy cut surface. The right ventricle was 0.7 cm. and the left 2 to 2.5 cm. in thickness. There were a few atheromatous plaques in the intima of the coronary

arteries, and a few small lipoid deposits throughout the length of the aorta.

Lungs: The lungs weighed together 1710 gm. The pleural surface of each lung was roughened by minute tubercle-like elevations measuring in diameter from a fraction of a millimeter to about 4 mm. These small nodules were more conspicuous in the lower lobes, especially on the diaphragmatic surfaces where they gave the pleura a finely granular appearance. The lower lobes of both lungs were firmly consolidated and a dark bluish red. On section the cut surfaces of both lungs had a granular appearance on account of the presence of many pale, translucent, wax-like nodules. These were easily dislodged from the tissue by the tip of a knife and appeared then as small tapioca-like granules. They were particularly numerous near the diaphragmatic borders, but were also seen in the lighter colored, more crepitant upper lobes. A fibrous and calcified nodule about 1.5 cm. in greatest dimension was found near the apex of the right upper lobe beneath fibrous pleural adhesions. A main branch of the pulmonary artery supplying the left lower lobe was distended by a firm but friable, reddish gray blood clot. The tracheobronchial lymph nodes were much enlarged, dark red and friable, because of the presence of many small granules similar to those in the lungs.

Spleen: The spleen weighed 160 gm., was firm and a bluish gray. The cut surface was of somewhat glassy appearance and the malpighian corpuscles were indistinct.

Gastro-Intestinal Tract: In the mucosa of the stomach and duodenum were several small superficial erosions about 0.3 to 0.7 cm. in diameter, a few of which had hemorrhagic margins. In the mucosa of the jejunum were several small thickened areas, some of which had yellow opaque centers, the largest being about 0.4 cm. in diameter. There were hemorrhages in the wall beneath the lesions.

Pancreas: Not remarkable in appearance.

Liver: The liver weighed 1600 gm. The peritoneal surface was slightly granular with numerous small yellow foci. The organ cut with moderate resistance and the sectioned surface had mottled yellowish or pink lobular areas with central red foci. The gallbladder was not unusual.

Adrenals: These were slightly larger than usual with a prominent translucent grayish medulla.

Kidneys: Each weighed 210 gm. and was firm and dark red. An excess of dark blood was scraped from the cut surface.

Bladder, Prostate, Seminal Vesicles and Testes: Not unusual.

Organs of the Neck: The thyroid and parathyroids appeared normal. A moderate amount of pinkish mucoid material was present in the trachea and larynx.

Lymph Nodes: The preaortic lymph nodes resembled the tracheobronchial lymph nodes already described. The mesenteric lymph nodes were somewhat enlarged, pale and friable.

Brain and Spinal Cord: Not examined.

Iodine Test: The iodine test for amyloid with Lugol's solution was employed on heart, lung, spleen and tracheobronchial lymph node. It was strongly positive on the heart and lungs, moderately positive on the spleen and only suggestive on the lymph node.

Microscopic Examination

Heart: Practically every muscle fiber was surrounded by a zone of light pink staining material, in part homogeneous and in part fibrillar, causing a separation of the fibers from one another. The deposit was more abundant in the left ventricle. In each ventricle the muscle fibers had shrunk away from the surrounding substance. Some of the fibers were increased in size but many were greatly decreased in diameter. The small capillaries were surrounded by the pink matrix and this material was found in the walls of small arterioles, especially in the media and adventitia.

Lungs: A pink staining hyaline material was seen in the walls of blood vessels of varying size, both arteries and veins. The amount varied, in some instances being so extensive that little remained of the original structure of the vessels but a few strands of elastic tissue recognizable in a section stained by the combined Weigert and Van Gieson methods. In a number of vessels the internal elastic lamella could be seen to end abruptly in the deposits. In many instances it was not possible to distinguish arteries from veins, because of the degree of alteration. The larger vessels were less changed than the medium sized and small ones. The substance was also found in varying amounts in alveolar walls, in some places producing only a slight thickening. This minimum degree of involve-

ment was particularly noticeable in sections from the upper lobes. In other areas there were large irregular or rounded masses of amyloid, whose relation to the alveolar walls was difficult to determine, even with the aid of the elastic tissue stain. In some of the masses small fragments of elastic tissue were recognizable as remnants of the alveolar septa. Delicate strands of red staining collagenous tissue were also seen in the more homogeneous, dull orange colored amyloid. Lumens of one or more blood vessels were frequently found in the masses. The alveolar lumens in places were greatly reduced in size on account of compression by the hyaline material and in many instances were lined by cuboidal epithelium. A few alveoli were dilated. The lumens contained a number of large mononuclear cells with brown pigment granules in their cytoplasm. In places there was proliferation of fibroblasts, apparently in the larger septa. The blood vessels were congested; in the lower lobes the alveoli were packed with red blood cells and in a few places contained small amounts of fibrin and numerous polymorphonuclear leukocytes. Amyloid was present in the walls of small bronchi in the sections, especially in the mucosal layers. There were a few multinucleated giant cells at the edges of amyloid masses or in crevices within them. In some instances the cytoplasm of these cells was poorly differentiated from the amyloid.

Spleen: The malpighian corpuscles were replaced to a varying degree by a pink staining hyaline material. In addition this substance was found irregularly distributed throughout the pulp. Lymphoid tissue was greatly reduced in amount and red blood cells formed masses in between the amyloid deposits.

Pancreas: In the walls of small arteries there was a small amount of amyloid.

Liver: Extensive congestion and hemorrhage were present in the central parts of the lobules, with necrosis of some of the cells. There was a slight increase of connective tissue in the centers of the lobules. In many of the portal spaces the walls of the vessels, especially the portal veins, contained pink staining material.

Adrenals: In the inner part of the cortex, and to a slight extent in the medulla, there was a small amount of homogeneous, light pink staining material between the cells. A few of the vessels in and about the adrenal had similar deposits in the walls.

Kidney: A slight thickening of the walls of the capillaries of some of the glomeruli was seen and there was hyaline material in the walls of a few small arteries.

Prostate: The interstitial tissue was somewhat increased due in part to small masses of hyaline pink staining material, especially noted about small blood vessels.

Intestine: In localized areas in the mucosa, and especially the submucosa of the jejunum, were hyaline deposits in and around small blood vessels.

Thyroid: In a few areas a homogeneous material was present in the interstitial tissue and the walls of small vessels were frequently hyaline in appearance.

Diaphragm: Amyloid was present in the walls of a few of the smaller arteries but only a trace of the material was present between the muscle fibers.

Lymph Nodes: Section of a tracheobronchial lymph node presented almost complete replacement of the usual structure by homogeneous material. The follicles had disappeared and the lymphocytes were scattered here and there. Small blood sinuses were embedded in dense tissue. Mesenteric lymph nodes presented a similar picture.

Cultures: Postmortem cultures of the heart's blood, spleen and lung were sterile. Three rabbits, three guinea pigs and several white mice were injected subcutaneously and intraperitoneally with emulsion prepared from lung tissue. None presented any significant anatomical findings at autopsy several months later.

Comment

In 1929 Lubarsch [11] described 3 cases in which the lungs were involved by amyloid. In 1 case there was extensive amyloid in the tongue, pharynx, esophagus and remainder of the gastro-intestinal tract, as well as in the heart and other organs, with the exception of the liver, spleen and kidneys. There were large amounts in the lungs, in many places in the form of solid nodules. In the 2nd case there was amyloidosis of the heart, tongue, esophagus and skin, with amyloid present only in the walls of arteries and veins of the lungs. In the 3rd case amyloid was abundant in the spleen, lymph nodes, prostate, seminal vesicles, epididymides, testes, stomach, intestines

and heart. There were "pinhead" to "lentil" sized nodules in the lungs. In the 1st and 3rd cases Lubarsch found no obvious underlying disease. In the 2nd case he believed that cystitis, pyelitis, furunculosis and recurrent valvular endocarditis could not be disregarded as possible factors. Physical signs suggestive of myocardial disease were present only in the 2nd case and were not of major significance.

Larsen [12] in 1930 cited the case of a man who had a typical history of failure of the left side of the heart, although death was due to a streptococcus septicemia. There were amyloid deposits in the heart and spleen and extensive diffuse involvement of the lungs. In the degree of involvement of the lungs and heart the 1st and 3rd cases of Lubarsch, and the case reported by Larsen, bear a fairly close resemblance to the 2nd case reported here.

There is another group of cases of atypical amyloidosis particularly with involvement of the heart and relatively insignificant involvement of the lungs. In 1886 Wild [13] presented a case of amyloidosis with especial involvement of the heart, but with some amyloid in the arteries and veins of the lungs and the mucosa and submucosa of the bronchi. There was no known predisposing disease. Clinically there was evidence of emphysema and weakness of the myocardium. Beneke and Bönning [14] in 1908 described the autopsy findings in an emaciated aged male who suffered from bronchitis. The clinical history was inadequate. Amyloid was present in the heart, and in small amounts in the lungs, but in no other organs. Koller [15] in 1932 presented a case of amyloidosis of the heart, tongue and bone marrow and small amounts of amyloid in the walls of the small blood vessels and capillaries of the lungs and in the alveolar septa. Diabetes was the only other disease present. Clinically there was cardiac insufficiency with edema of the lungs.

Three unusual cases of amyloidosis were reported by Bannick, Berkman and Beaver [16] in 1933. In only 1 of these cases were the lungs affected. There was generalized amyloidosis including involvement of the spleen, liver and kidneys. The walls of some pulmonary alveoli and of many blood vessels contained amyloid. The myocardium was somewhat friable and waxy, and microscopically showed a moderate amount of interstitial amyloid with atrophy of some of the muscle fibers. There were no cardiac symptoms. The etiology of the deposits was obscure.

In the same year Strauss [17] described atypical amyloidosis with particular involvement of the lymph nodes. Considerable amyloid was seen in the lungs in the large and small septa, and in the walls of the bronchi and blood vessels. There was considerable amyloid in the epicardium and endocardium but somewhat less in the myocardium. Symptoms of cardiac failure were not present.

Perla and Gross [18] in 1935 reviewed 112 cases of amyloidosis found in a series of 1500 autopsies. Three were considered as atypical, and in 1 of these, a patient with signs and symptoms of congestive heart disease, small amounts of amyloid were present in the lungs. The heart, tongue, kidneys and gastro-intestinal tract were more extensively involved.

The most recent example in the literature is the case reported by Reimann, Koucky and Eklund [19] in 1935. The patient had had low grade fever, progressive weakness, loss of weight, swelling of the tongue and evidences of cardiac failure for several months. Amyloid was prominent in the medium sized arteries of all the tissues examined. In the heart it was also present in the interstitial tissue with atrophy of many of the muscle fibers. Small plaques or beads of the substance were present in the alveolar walls of the lungs.

Primary amyloidosis of the heart without amyloidosis of the lungs has been described by Steinhaus,[20] Kann,[21] Beneke,[22] Warren,[23] Brocher,[24] Humphreys [25] and Budd.[26] It is noteworthy that symptoms of cardiac failure were noted in only 3 of these. Brocher's case was unusual because of the striking diminution of the caliber of the branches of the coronary arteries on account of the deposits of amyloid in the walls with resulting necrosis of the myocardium. Amyloid was not found between the muscle fibers of the heart.

Discussion

In Case 1, carcinoma of the bladder, chronic infection of the kidneys, and a localized bronchiectasis with suppuration may each have played a part in the etiology of the amyloidosis. This case, therefore, cannot be considered as an example of primary amyloidosis of the lungs. It is noteworthy that in two instances (Herxheimer,[7] and Hallermann [10]) localized deposits of amyloid were noted in the lung in association with an adjacent empyema. The amyloid in Case 1 was intimately associated with the area of suppurative bronchiecta-

sis which may have been directly responsible for its formation. The lack of involvement of other organs with the exception of the spleen was an unusual feature.

In Case 2, there was no evidence of any associated disease such as tuberculosis, syphilis, prolonged suppurative inflammation or extensive neoplasm. Perla and Gross [18] have suggested on the basis of the experimental work of others that a dietary peculiarity might play a rôle in the etiology of certain cases of atypical amyloidosis. As far as we have been able to ascertain from the history and postmortem findings there was nothing unusual about this patient's diet. The amyloid deposits and the changes which they brought about were the only significant anatomical lesions. While amyloid was present in the spleen in considerable quantities the liver was little affected and the kidneys practically not at all. The organs unexpectedly involved were the heart and lungs. In the former the deposits were as extensive as those in any case we have found described in the literature, and undoubtedly in combination with the lesions in the lungs were the cause of the congestive heart failure. This clinical picture of myocardial failure with passive congestion of the organs and accumulation of fluid in the tissues and body cavities, produced by myocardial amyloidosis, has been observed by several writers and discussed, in particular, by Koller.[15] It is of interest that he noted also auricular thrombi, as did Larsen [12] and Perla and Gross.[18]

The presence of nodular deposits of amyloid in the lungs was a conspicuous feature in both cases described in this paper, although there were no masses of the size observed in the cases reported where amyloid was localized in the lungs. The unfixed heart and lungs in Case 2 reacted with a strong dark brown color to iodine, while a bronchial lymph node gave a much weaker reaction. Sections of paraffin embedded material from the lungs and several organs were stained with iodine, methyl violet, gentian violet, and Mallory's anilin blue stain. The amyloid was so faintly stained by iodine and by methyl violet that these methods had little value. In a frozen section of the lung stained with methyl violet the amyloid took a deeper lilac color. In paraffin sections the same reaction was seen with gentian violet, but the amyloid was not always well differentiated from other tissues. With the anilin blue stain the same difficulty was encountered. The amyloid, as well as the col-

lagen, stained a bright blue color. The combined Weigert's elastic and Van Gieson's connective tissue stain of the lung was the most useful in differentiating the various tissue elements in each case. The fact that alcohol was not used as a fixative may partly explain the unsatisfactory results with some of the methods used for demonstration of amyloid.

Let us now consider some of the features of pulmonary amyloidosis noted by other authors. As Kschischo,[4] among others, has pointed out, amyloid disease of the lungs may be divided into the localized form and that occurring in association with general amyloidosis. Examples of the former condition have been recorded by Herxheimer,[7] von Werdt,[8] Meyer,[9] and Hallermann,[10] while the other cases reported, including 2 presented here, belong in the second group, although in Case 1 the only other organ involved was the spleen. It is noteworthy that while the amyloid may be fairly widespread throughout the body, involvement of the liver, spleen and kidneys is seen in only about one-half of these cases. The heart is the site of amyloid in about the same number of instances, although the degree of involvement varies from slight deposits in the vessels to extensive interfibrillar masses.

From the standpoint of an obvious etiological factor we have again two groups. In the first of these there is no associated condition which would generally be considered as bearing a causal relationship. Most of the cases, including the 2nd one here reported, are examples. However, in the second group are the cases of Lubarsch and Plenge,[6] Herxheimer,[7] Schilder,[2] Kschischo,[4] Glaus,[5] Hallermann,[10] and Case 1, in which there is a presumably associated etiological disorder. Of these cases, 3 showed chronic pulmonary tuberculosis, 2 empyema, 1 multiple myeloma, 1 chronic inflammation of the lung, perhaps syphilitic, and 1 carcinoma of the bladder and localized bronchiectasis. It is noteworthy that in only 1 of these cases (Herxheimer's) was the amyloid limited to the respiratory tract. Here there was an associated empyema.

A study of the anatomical changes described by some of these authors reveals features of interest. Where the amyloid was grossly visible it was usually described as being homogeneous or hyaline, glistening or transparent gray or grayish yellow, and brittle. It was in the form of small nodules, streaks, or larger masses. In some instances an association with blood vessels or bronchi was observed.

The substance was distributed quite uniformly throughout both lungs or limited to isolated nodules or masses several centimeters in diameter in one or both lungs.

The lesions observed microscopically deserve consideration. The diffuse type, with which we are particularly concerned, varied from the scant deposits observed in the vessels and alveolar septa by Virchow,[1] to the extensive widespread amyloid degeneration of the lungs found in the 2nd case reported here. In almost all instances there was amyloid in the walls of the pulmonary vessels of small and medium size, involving especially the media. There was variation in the degree of participation of the capillaries of the alveolar walls, some authors having described amyloid occurring about them, while others claimed they were unaffected. Two or three writers mentioned an apparent involvement of the walls of lymphatics. Observations differed as to the participation of the alveolar walls. Some authors described the amyloid as being deposited in the connective tissue, while others, especially Schilder[2] and Kschischo,[4] found it beneath the lining epithelium in the form of fine streaks or rings. In other cases, described by Lubarsch,[11] Schilder,[2] Kschischo,[4] Glaus,[5] Larsen,[12] and Strauss,[17] the walls were so thickened by the amyloid deposits that the alveolar lumens were reduced in size, in some instances to mere slits. This change occurred in the cases here described. A cuboidal shape of the alveolar lining cells has occasionally been noted and in Kschischo's[4] case gave a glandular appearance to the lung. Involvement of the bronchi was observed by several authors, and in fact in Wild's[13] case the bronchi were affected while the alveolar walls were unchanged. The mucosa was usually involved and the amyloid was sometimes found around the mucous glands. Multinucleated giant cells were noted in close proximity to the deposits of amyloid by Herxheimer,[7] Meyer,[9] and Strauss.[17] The first two of these authors stated specifically that these giant cells did not contain any amyloid.

The two sexes were practically equally represented among the cases. The age distribution is of some interest. Aside from two individuals, 21 and 24 years old, both of whom had chronic pulmonary tuberculosis, all of the cases of amyloidosis of the lungs were in persons over 41 years of age.

There is no adequate explanation for the participation of the lungs in the changes of generalized amyloidosis in some cases, and their

lack of involvement in others. Most cases of advanced pulmonary amyloidosis may be considered as examples of atypical or primary amyloid disease, but in a number of instances amyloid has occurred in the lungs when there has been a characteristic generalized amyloidosis. Indeed, Lobel and Ten Thije[27] state that amyloid in the lung is not infrequently associated with amyloid in other organs. It seems highly probable that the minor grades of the condition are often overlooked or given but little attention. A small group of cases shows that advanced pulmonary amyloidosis without the usual precursors of this condition, especially when combined with extensive amyloidosis of the heart, may produce a fatal condition.

Summary

Amyloidosis of the lungs associated with general amyloidosis was first described by Virchow in 1857. Since that time a number of cases of pulmonary amyloidosis have been reported in some of which the deposits in the lungs have been part of a generalized amyloidosis, while in others the amyloid has been restricted to the lungs. In several instances these deposits were in the form of isolated nodular masses. Two new cases of amyloidosis of the lungs are reported here. In 1 of these, isolated deposits of amyloid were found in one lung, with more extensive amyloidosis of the spleen. A carcinoma of the bladder and localized bronchiolectasis of the affected lung were possible etiological factors. In the 2nd case there were unusually large quantities of amyloid in the lungs and heart and smaller amounts in many of the other organs. This case is an example of "atypical" primary amyloidosis since there was no recognized associated disease.

Conclusions

1. Amyloid deposits in the lung are scant or absent in most cases of generalized amyloidosis, and occur about blood vessels or within the alveolar walls.

2. In some instances amyloid deposited in large amounts, frequently in nodular form, greatly alters the pulmonary architecture.

3. In most of such cases there is no preceding or concurrent chronic disease usually regarded as the precursor of amyloid.

4. Advanced amyloidosis of the lungs and heart may produce cardiac failure and death.

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DESCRIPTION OF PLATES

PLATE 126

FIG. 1. Case 2. Gross photograph of the lungs, showing nodules of amyloid, especially in the lower lobes, with other large areas of homogeneous appearance.

FIG. 2. Case 2. The heart shows increased thickness of the walls and mural thrombi in the auricles.

1

2

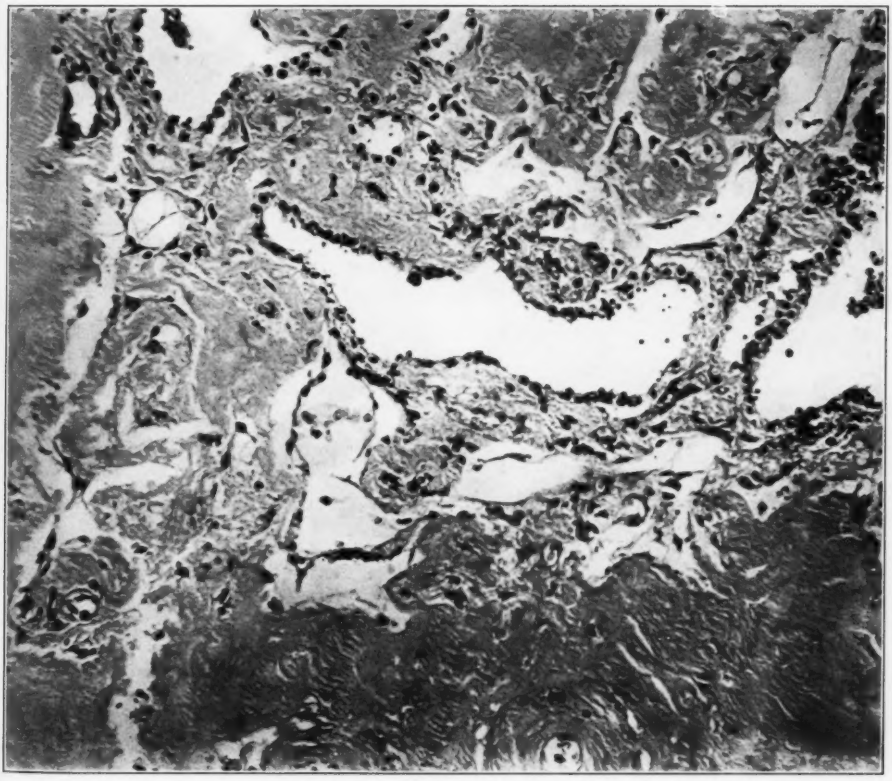
Ferris Amyloidosis of Lungs and Heart

PLATE 127

FIG. 3. Case 2. Photomicrograph of lung showing amyloid in alveolar walls and small arteries. × 265.

FIG. 4. Case 2. Large masses of amyloid with partial or complete obliteration of alveoli. There is cuboidal metaplasia of the lining epithelium. × 220.

3



4

PLATE 128

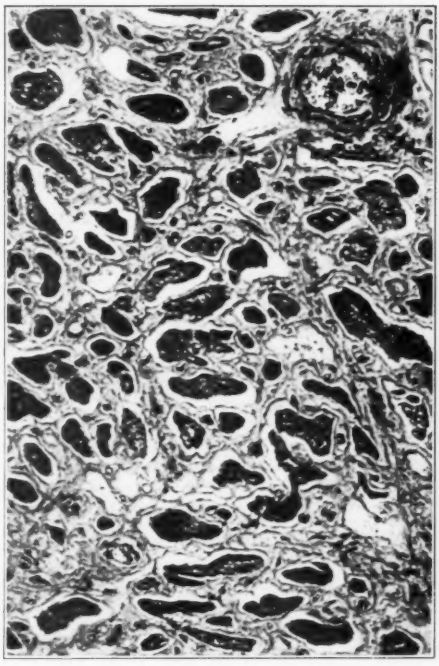
FIG. 5. Case 2. Amyloid masses with multinucleated giant cells in lung. × 250.

FIG. 6. Case 2. Left ventricle with muscle fibers widely separated by amyloid, appearing for the most part as fine linear deposits. × 220.

FIG. 7. Case 2. Amyloid in a malpighian corpuscle of the spleen. × 270.

FIG. 8. Case 2. Tracheobronchial lymph node. Amyloid has largely replaced the lymphoid tissue and distorted or obliterated the lymph sinuses. × 160.

5



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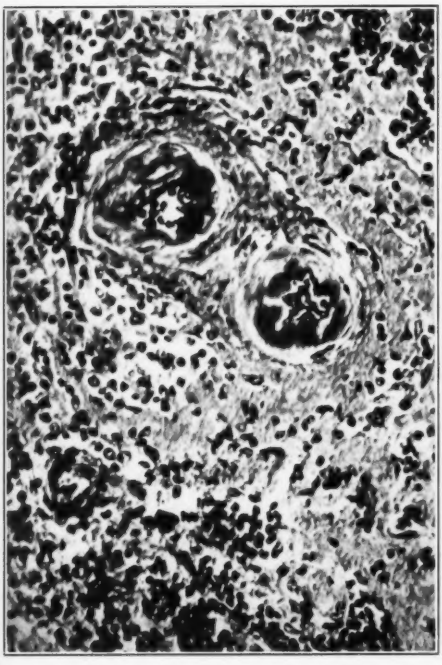

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8

Ferris

Amyloidosis of Lungs and Heart

SCIENTIFIC PROCEEDINGS OF THE THIRTY-SIXTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

HELD AT HARVARD MEDICAL SCHOOL,

BOSTON, MASSACHUSETTS,

APRIL 9 AND 10, 1936

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

President WOLBACH in the Chair

The Secretary presented the nomination of the Council for officers as follows:

President	N. CHANDLER FOOT
Vice-President	E. B. MCKINLEY
Treasurer	F. B. MALLORY
Secretary	HOWARD T. KARSNER
Incoming Member of Council	STANHOPE BAYNE-JONES
Assistant Treasurer	FREDERIC PARKER, JR.
Assistant Secretary	ALAN R. MORITZ

Voted unanimously to elect those nominated.

Voted to elect the following new members:

Frank L. Apperly	Mendel Jacobi
Roger D. Baker	Abraham R. Kantrowitz
Howard A. Ball	Edwin H. Lawson
S. S. Blackman, Jr.	Max Lederer
Clark E. Brown	James R. Lisa
John W. Budd	Elizabeth Moore
Caspar G. Burn	David R. Morgan
Benjamin Castleman	Charles Oberling
John J. Clemmer	H. D. Palmer
G. Lyman Duff	Joseph G. Pasternack
Frederic H. Foucar	James H. Peers
Isadore E. Gerber	Joseph E. Pritchard
Harry S. N. Greene	Walter Putschar
Robert E. Gross	John R. Schenken
Bela Halpert	Arthur Schifrin
J. Beach Hazard	Martin Silberberg
Frederick A. Hemsath	Morris A. Simon
John D. Houghton	Joseph E. Smadel
Dudley A. Irwin	Lawrence Sophian

Paul E. Steiner	Phillips Thygeson
Ward W. Summerville	Arthur J. Vorwald
Joseph Tannenberg	Ruth C. Wanstrom
E. T. Thorsness	Frederick W. Wiglesworth

Sigmund L. Wilens

It was also voted to reinstate Drs. C. L. Connor, F. E. Dolman, J. J. Moore, S. S. Schochet, and W. A. Starin.

Voted to accept with regret the resignations of Drs. R. L. Benson, A. E. Boycott, B. C. Crowell, F. T. Fulton, C. H. Hitchcock, A. P. Hitchens, A. Meyer, F. G. Novy, M. J. Rosenau, and W. C. Thro.

Voted to record with deep regret the deaths of Drs. G. C. Huber, R. V. Lamar, E. R. LeCount, and C. Norris.

Voted to nominate Dr. E. B. McKinley as representative of this Association in the Division of Medical Sciences, National Research Council.

Voted to adopt as the topic for the Symposium in 1937, The Genesis of Tumors. It was voted to appoint four referees and to request each one to present a paper limited to 20 minutes.

Dr. C. V. Weller is to be requested to talk on Heredity

Dr. Leo Loeb on Sex Hormones

Dr. S. Burt Wolbach on Immediate Response to Carcinogenic Chemicals

Dr. Peyton Rous on Filterable Agents

Voted to accept the invitation of Northwestern University Medical School to meet in Chicago in 1937, on Thursday and Friday, March 25 and 26, 1937.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

INFLAMMATION IN RESISTANCE TO INFECTION. Arnold Rice Rich, Baltimore, Md.

Abstract. A well developed resistance to infection, whether native or acquired, manifests itself in three fundamentally important ways: (1) the bacteria are prevented from spreading freely through the tissues from the site at which they lodge; (2) the progressive multiplication of the bacteria is inhibited, and most or all of those that penetrate into the tissues are destroyed; and (3) any injurious substances that the bacteria are capable of liberating either before or after their death are rendered less noxious, or even completely harmless. This communication deals with a consideration of the manner in which some of the constituents of the inflammatory exudate contribute toward the accomplishment of these ends.

The increased flow of fluid from the blood vessels into the inflamed tissues serves to dilute injurious bacterial products, and in the immune body the exuding fluid may contain specific antitoxin, opsonin, lysin, and also antibody that effects the local precipitation of soluble injurious bacterial substances. The increased fluid movement may exert untoward effects by sweeping bacteria from the site and so spreading the infection; and when edema is marked the bacteria may proliferate progressively because they are out of reach of leukocytes, which cannot swim effectively through fluid.

The clotting of the fluid exudate serves a beneficial purpose in that the fibrin so produced provides a framework on which the leukocytes can crawl toward the bacteria. A second important function of the fibrin is that it inhibits the spread of the bacteria, though it is not yet clear that this effect is a simple mechanical one. It is possible that physicochemical conditions play a rôle in causing the bacteria to adhere to the fibrin and to the inflamed tissues.

In the body with acquired immunity, antibody plays an important rôle in inhibiting the spread of the bacteria. Antibody alters the physical characteristics of the bacterial surface in a manner that causes the microörganisms to adhere to each other (agglutination) and to the phagocytes (opsonization); and in like manner they are caused to adhere to the tissues at the site of infection and are thus prevented from spreading to infect distant tissues. This immobilizing action of antibody acts independently of any mechanical fibrin barrier and constitutes one of the highly important protective mechanisms of immunity.

Other protective potentialities of the inflammatory fluid are discussed, and the need for a more active investigation of the relation of the physicochemical conditions in inflamed areas to the survival requirements of different pathogenic bacteria is stressed.

The ability of the body to suppress the growth and to destroy the invading bacteria is another very important factor in the localization of infection. Certain strains of Type III pneumococci, for example, produce large local lesions in normal rabbits, but recovery always occurs although the phagocytes of the normal rabbit cannot ingest these bacteria. Miss McKee and I have found, however, that within the astonishingly short time of a few hours after infection the

animal has already acquired an increased ability to destroy the bacteria, and this reaches a marked degree of efficiency at the end of 24 hours, at which time the bacteria, if injected at a different site, can produce only a small lesion before they are destroyed. This observation has an obvious bearing not only on bacterial localization but also on the mechanism of native immunity and recovery from certain infections. Our *in vivo* and *in vitro* studies have shown that in this particular instance the fever developing after infection plays a highly important rôle in bringing about the destruction of the bacteria.

Concerning the cellular part of the exudate, while the macrophage is clearly the principal defense cell in certain infections (*e.g.* tuberculosis) there is no acceptable evidence that it overshadows the polymorphonuclear leukocyte in importance in resistance to pyogenic infections, as has been claimed by some. Nor is there any evidence for the existence of an acquired "fixed tissue immunity" that can preserve the body from bacterial attack independently of the leukocytes. Thus, if animals are highly immunized and then deprived of their phagocytes, pneumococci will grow progressively in their tissues and overwhelm the body, even though the body fluids are rich in antibody and potent in passively protecting animals possessing leukocytes. This observation fails to support the hypothesis that protective antibody exerts a direct bactericidal action *in vivo* in types of immunity in which no bactericidal action of antibody can be detected *in vitro*.

Our complete ignorance of the function of the lymphocytes is a serious gap in medical knowledge. Experiments are described which appear to point toward at least one function of these cells. It is shown that the first detectable morphological reaction of the body to bacterial or non-bacterial foreign protein that enters the blood stream is a proliferation and marked cytological alteration of the lymphocytes. The possible relation of this cellular activity to antibody formation is discussed.

It has long been believed, particularly in the case of tuberculosis, that the hypersensitive state is necessary for the operation of acquired resistance. Experimental evidence is presented which demonstrates clearly that such is not the case, whether in tuberculosis or in any other infection so far studied (syphilitic, pneumococcal, streptococcal, and *Pasteurella* infection). It is shown that in none of these infections is allergic inflammation necessary for protection since (1) acquired immunity can be established without the concomitant development of hypersensitivity; (2) a high degree of hypersensitivity can be established without a concomitant development of increased resistance; (3) the immunity of an immune, hypersensitive animal can be transferred to a normal animal without the concomitant transfer of hypersensitivity; and (4) when the hypersensitivity of an immune, hypersensitive animal is abolished by desensitization, the immunity remains intact. Since no experiment has ever been placed on record in which allergic inflammation has been shown to be necessary for protection in any infection, it is advisable to refrain from attributing to hypersensitivity any protective effect observed in the immune body until a test has been carried out to determine whether or not the same effect will occur in the absence of hypersensitivity. The need is stressed for the development of better methods of desensitization, for the purpose of freeing the body from the damaging effects of hypersensitivity in appropriate cases in tuberculosis and in other bacterial infections.

LOCAL ACIDOSIS IN INFLAMMATION. Valy Menkin, Boston, Mass.

Abstract. Earlier studies by the writer (*Am. J. Path.*, 1934, **10**, 193–210) have shown that the cytological sequence in acute inflammation, which consists of an initial polymorphonuclear leukocytic phase followed by an infiltration of mononuclear phagocytes, seems to be conditioned by the changes in the local hydrogen ion concentration. With the developing local acidosis in an acutely inflamed area the pH of the exudate drops from about 7.3–7.4 to 6.7–6.5. Polymorphonuclear cells for the most part seem unable to survive, without being injured, a pH below 7.0. From pH 7.0 down to 6.8 mononuclear phagocytes predominate. At greater hydrogen ion concentration, as found in frank pus, all types of leukocytes appear markedly injured. When the pH is alkaline, polymorphonuclear cells are found in greater abundance. By determining the pH the expected cytological picture of an inflammatory exudate can be predicted, and *vice versa*.

The present study was undertaken with the collaboration of my associate, Mrs. C. Warner, in an endeavor to determine the mechanism conducive to the production of local acidosis in inflammation. The elucidation of this mechanism would probably be of distinct aid in an understanding of the factors controlling the histological manifestations of a variety of infectious lesions. Earlier work of the writer demonstrated that the circulation in an acutely inflamed area is impaired by the formation of thrombi occluding small vessels (*Arch. Path.*, 1931, **12**, 802–828). Lymphatic blockade was traced as one of the earliest phenomena in acutely inflamed areas. With the progress of the inflammatory reaction many of the small vascular channels likewise become occluded by hyaline thrombi. The area of inflammation becomes shunted off, as it were, from the rest of the organism. It develops its own hydrogen ion concentration, metabolism and local circulation. Studies, with the aid of Miss H. Blatt of the Children's Hospital, have revealed that the alkali reserve, as determined by changes in volume per cent of CO_2, falls in an inflamed area parallel both with the drop in pH and with the replacement of polymorphonuclear leukocytes by mononuclear phagocytes. The depletion in the alkali reserve would account for the production of an uncompensated acidosis at the site of inflammation. Lactic acid has been found to be a constant constituent of pus (T. Irisawa, *Ztschr. f. Physiol. Chem.*, 1893, **17**, 340–352). The relative anoxemia, which must doubtless exist in an area of impaired local circulation such as occurs with the progress of the acute inflammation, suggests the possibility that the local acidosis may be referable to increased anerobic glycolysis. It is, however, to be remembered also that leukocytes *per se* display pronounced glycolytic activity. The conversion of sugar to lactic acid in acute inflammation may well explain the rise in hydrogen ion concentration and the accompanying cellular change. Studies on pleural inflammation in dogs revealed that this is precisely the mechanism involved. As the pleural inflammatory reaction progresses in intensity, the rate of glycolysis increases two to four-fold. The sugar level falls markedly and the hydrogen ion concentration rises. The concentrations of lactic acid, hydrogen ions and sugar in the blood stream remain essentially unaltered. The low lactic acid in the circulation and the high blood sugar indicate a distinct reciprocal relationship in comparison with the concentrations of these substances in the exudate. The studies show that the cytological picture in inflammation is evidently referable to the local hydrogen ion concentration, which in turn is primarily conditioned by the rate of glycolysis and the local depletion of the alkali reserve. The implications of these facts in regard to various histological lesions are obvious and

are accordingly being studied further. Preliminary studies on tuberculous lesions in lungs of infected rabbits, where the mononuclear phagocyte constitutes the predominating cell type, reveal a high rate of glycolytic activity. Normal rabbit lung, on the contrary, is characterized by a high sugar concentration in its tissue, in comparison with the quantity of lactic acid recovered.

The inflammation induced by *Staphylococcus aureus* in the skins of rabbits reveals in the first few hours of the reaction a normal glycolytic activity. Within 2 hours after the inoculation of the organisms, lymphatics at the site of inflammation are found distended and containing many fibrinous plugs. When the reaction has been in progress for 10 hours a definite increase in glycolysis is observed. The polymorphonuclear cells still predominate, showing that the increased glycolytic activity precedes the cytological change. In staphylococcus inflammation of 1 days duration the abscess is fully formed and contains in its core an abundance of degenerated polymorphonuclear cells. Normal mononuclear phagocytes predominate, and there are present also a number of injured mononuclears. The glycolytic activity is very high. At later stages frank pus is observed and most of the cells in the abscess are either dead or degenerated. These observations suggest that the mechanism of suppuration is in large part basically referable to an increased glycolytic activity at the site of acute inflammation. The resulting uncompensated acidosis prevents the survival of leukocytes.

Discussion

(Dr. Warfield T. Longcope, Baltimore.) My information about this important subject is very meager, but I was impressed by the observations that Dr. Rich has made on the fixation of bacteria in the tissues at the site of inflammation, and I was much interested in the probable explanations he developed to account for this process, which undoubtedly plays an important part in preventing rapid spread of the local infection. Another matter of which he spoke and which is quite new to me concerns the differences in the reaction of the skin which were observed on the repeated subcutaneous injections of *Pneumococcus* Type III in rabbits. He mentioned the possibility that an elevation of the temperature of the rabbit might account for the fact that the 2nd and 3rd subcutaneous injections, made 24 to 36 hours after the primary injection, resulting in smaller and smaller lesions, might be due to the increased body temperature of the rabbit. I should like to know whether he believes that this alone accounts for the variations in reaction which he has observed, or whether it is conceivable that still other factors might influence this interesting phenomenon.

(Dr. Francis G. Blake, New Haven.) There is one point of interest brought out by Dr. Rich's experiments on Type III *Pneumococcus* in the rabbit. The point I wish to make is that the experiments illustrate so well the danger of drawing conclusions about inflammatory phenomena in a given animal when one test has been made 24 hours before another. There have been many experiments in the literature, for example, in which preliminary skin tests for hypersensitiveness have been done to determine the state of hypersensitiveness of the animal 24 hours before an infection has been established. Dr. Rich's results should warn us very clearly of the fact that even small preliminary doses of antigen to test for hypersensitiveness may very easily modify the course of an infection initiated 24 hours later.

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask Dr. Rich in closing his discussion to state whether he has found an explanation of the mechanism of

hyperemia which initiates the inflammatory reaction; also whether he has any evidence on the mechanism by which leukocytes are attracted to the area of inflammation.

(Dr. Marion B. Sulzberger, New York City.) Dr. Rich, if I understood him correctly, said it has long been the general belief that the reaction of hypersensitivity, which he called "allergic inflammation," was a favorable reaction, and one beneficial to the patient or infected organism. I think dermatologists who have been interested in immunology have long since abandoned that concept. They recognized many years ago that an acute inflammatory allergic response is of itself not necessarily beneficial but, on the contrary, often destructive. This conclusion was forced on them by observations in such diseases as skin tuberculosis and syphilis. For precisely at the time when the syphilitic process is most destructive to bone and skin, the luetin reaction demonstrates the existence of the highest degree of hypersensitivity. And precisely at the time when the tuberculous process is most destructive, the reaction to tuberculin is likely to be greatest. On the contrary, dermatologists studying the sarcoid forms of tuberculosis have found that the immunity to tubercle bacilli is particularly well developed in just those cases in which there is an acquired *anergy*, a marked lack of response to tuberculin. This is illustrated in the form of tuberculoderms which are not destructive, in which the bacilli are absent or disappearing, and in which the tuberculin reaction is not an allergic hypersensitivity but a specifically acquired (and therefore allergic) *hyposensitivity*. These considerations are beautifully illuminated by the recent experiments of W. Jadassohn. These studies were carried out on rats, animals to a high degree immune to bovine and human strains of tubercle bacilli. When one injects rats with virulent tubercle bacilli this generally produces no reaction of tissue hypersensitivity. There is no hyperergic inflammatory response, no fibrin occlusions; and these animals do not become tuberculin hypersensitive, for they continue to have a negative tuberculin reaction in spite of the infection. It is in this connection noteworthy that the rat is one of the animals possessing *a priori* a high titer of tuberculin neutralizing antibodies in its blood stream (natural antibodies?). I believe that these clinical and experimental examples, which are but a few of a vast number of similar observations, will suffice to show that dermatological experiments and observations during the last 20 years led to conclusions essentially in general accord with Dr. Rich's present concept. Furthermore, the method which he suggests for immunization has been practiced constantly in those tuberculoderms in which there is hypersensitivity to tuberculin; and quantitative, gradual immunization can frequently be carried out successfully, and the sensitivity to tuberculin reduced, or even abolished. And when this tuberculin sensitivity is reduced the tuberculoderms usually disappear. So that we may say that with the deliberate reduction in the hyperallergic response, there is an increased immunity to the acquired infection. This holds true in other dermatological conditions—for instance, in the treatment of mycotic affections by means of trichophyton hyposensitization.

I should like to refer those interested in this subject to J. Jadassohn's Handbuch der Haut und Geschlechtskrankheiten, Volumes II, X, and XI. Here they will find not only excellent discussions of these problems and experiments, but also a fairly inclusive bibliography of the voluminous and pertinent literature, which should prove interesting to those who are, like Dr. Rich, continuing in this splendid and important work.

(Dr. Jules Freund, New York City.) I think Dr. Rich's observation on *Pneu-*

mococcus Type III is most significant, regardless of whether it is specific or nonspecific, and I should like to ask whether the protection that develops within one day is specific or not.

(Dr. N. Chandler Foot, New York City.) Dr. Rich has just stressed our ignorance concerning the lymphocyte; there is another cell, regularly a constituent of Hodgkin's granuloma, also seen in continued chronic inflammation, the eosinophilic polymorphonuclear. I should like to ask if he has any data on the rôle and reason-for-being of the eosinophilic polymorphonuclear.

(Dr. S. Burt Wolbach, Boston.) Why don't you include also the mast cells?

(Dr. Stuart Mudd, Philadelphia.) Dr. Strumia will report this afternoon that the eosinophile is also phagocytic, although to a less degree than the neutrophile.

I should like to ask if Dr. Rich would say a word on the type of evidence that Dr. Dienes has been bringing forward, indicating that allergic irritability may be a first stage in the process of active immunization, and possibly a stage that has to be gone through in order to reach a stage of active immunity.

(Dr. L. Dienes, Boston.) I believe that one of the obstacles in the study of bacterial allergy was that the strong necrotic skin reactions were regarded as characteristic of it. If the quantitative conditions of the skin reactions are considered, it is apparent that these strong skin reactions are purely artificial phenomena comparable to acute anaphylactic shock. A tuberculous guinea pig rarely gives a necrotic skin reaction to less than 0.02 mg. of tubercle bacilli. This amount corresponds to about 10,000,000 bacilli. In the natural course of the disease such amounts of bacteria are not distributed in the tissues. Other animal species are less sensitive than guinea pigs. In slight skin reactions tissue destruction is absent and a productive inflammatory reaction is characteristic of them. At the injection sites a strong cellular infiltration develops in which large mononuclears predominate, and if tubercle bacilli are injected the formation of tubercles is accelerated. This tissue response is not characteristic of tuberculosis. It is characteristic of the first active stage of immunity response before free antibodies appear in the circulation. It is observed in animals injected with egg-white or horse serum as well as in tuberculous animals, if they are skin-tested with the specific antigen 3 to 6 days after the first injection. In certain diseases this active stage of immunity response is strongly developed and the defensive reaction is mainly a tissue reaction. In other diseases the humoral antibodies seem to play a more important rôle. The study of active immunity process in the tissues presents more difficulties than the study of antibodies. This process cannot be passively transferred from one animal to the other like antibodies. In most cases it cannot be reproduced by vaccination, though it is strongly developed in the actual disease. For this reason its significance in the disease is not sufficiently known. Bacterial allergy is not an exception in this respect and the significance of most processes which take part in the mechanism of healing is not sufficiently known. A process like bacterial allergy, which is closely connected with the development of immunity, and which is known to exert a strong influence on the tissue response, certainly deserves our attention even if at present, in some cases, its influence appears harmful.

(Dr. Everitt G. D. Murray, Montreal.) I should like to try to contribute some personal observations which might be used to study these polymorphonuclear and large mononuclear cells. In inoculating *Gastrophilus intestinalis* larval salivary glands into the rabbit's pleural or peritoneal cavity we get an enormous exudation of leukocytes and the predominating cell is the polymorphonuclear neutrophile, but within 5 to 6 hours there are large numbers of macrophages.

This salivary gland extract injures the polymorphonuclear neutrophile, as is evidenced by the marked phagocytosis of the polymorphonuclears by the macrophages. This gland extract represents a small amount of material, and if you increase the activity of this glandular material by acidifying it with N/100 hydrochloric acid, so that you are injecting a definitely acid material, the exudation of polymorphonuclears is just as great as though you injected a neutral material, but they are still much injured and the macrophages devour them just as readily. These larvae are very common in the stomachs of horses. The dissection of the salivary gland is simple, and can be done with sterile precautions, and it has this curious specific stimulation for the accumulation of these cells. If you take the *Gastrophilus* larvae and dissect out their alimentary tract you find not red blood cells in it, but leukocytes (as observed by Keilin), so that it looks as if these larvae have the ability of selecting the food which they most like to eat by virtue of their salivary secretion.

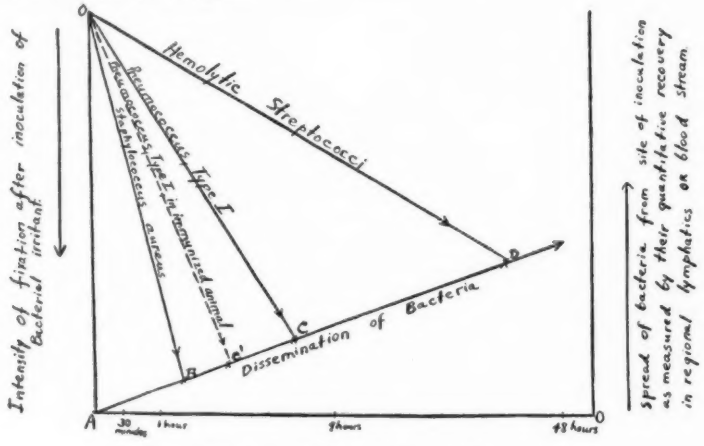
(Dr. Menkin, closing.) In regard to the comment made on the effect of salivary gland extracts in inducing inflammatory reactions, this is a very interesting observation. I should like to reemphasize the fact that the injurious effect on the polymorphonuclear cells of the exudates studied was found when the pH of the exudate fell below 7.0. Concerning modification of the pH by injections of various substances, such as HCl, we have encountered considerable difficulties. I have tried to inject acid phosphate buffers prepared by the Sorenson-Cohn method in an endeavor to induce an acid pH in our inflamed areas and thus prevent the development of the initial polymorphonuclear phase. We have, however, not succeeded in obtaining any satisfactory results. The cytological sequences of inflammation with the usual hydrogen ion changes from an alkaline pH to an acid pH proceeded just the same. The injections of derivatives of iodoacetates which *in vitro* prevent the formation of lactic acid have thus far failed to do so *in vivo*. As is well known, *in vitro* studies are not always reproducible *in vivo*. In other words, it seems to me that we know as yet very little about the mechanism and control of the tissue buffer system, even though considerable information exists concerning the buffering system of the blood. Thus far we have been unable to modify the course of the hydrogen ion concentration in acute inflammation except to accelerate or intensify the process. However, this question is being investigated further. There is also the possibility of a certain amount of specificity in the effect of the acid on leukocytes. We are at present studying the effect of lactic acid on the leukocytes of an exudate *in vitro*.

In regard to the interesting work of Dr. Rich, I should first like to state that there is essentially no conflict of views between Dr. Rich and myself. Dr. Rich has effectively studied the reactions in immunized animals, whereas I have devoted my attention primarily to the mechanisms concerned with the non-specific type of inflammation. At first we were interested in answering this basic question: What is the capacity of the local inflammatory reaction in retarding the dissemination of various substances and bacteria to the regional lymphatics or to the circulating blood? The question is simple, and it seems to me that it has been answered by a large amount of experimental evidence. In brief, severe inflammation definitely tends to delay the dissemination of various substances, including bacteria, from its focus. The primary mechanism responsible for this local fixation is the presence of a biological barrier in the form of coagulated plasma and thrombosed lymphatics at the site of inflammation. The evidences obtained indicate that this mechanical barrier can by itself effectively and primarily account for the retardation in the dissemination of colloidal substances, particulate material, or of bacteria from the site of inflammation. As has often been pointed

out previously, this does not, however, preclude the involvement of other secondary factors, such as anatomical position of the inflamed area or interaction of some of the substances tested with exudate followed by the precipitation, agglutination, or coagulation of the former. These factors may in some cases reinforce the primary mechanism of fixation.

Concerning the question of the *immediate* spread of the bacteria at the beginning of the inflammatory reaction, there is little doubt that some, though not all, of the microörganisms, as indicated by direct observations, can disseminate at once from the site of inoculation. From the standpoint of the non-specific inflammatory reaction there is really nothing to restrict the dissemination of some of the bacteria *immediately* after their inoculation and prior to the development of lymphatic blockade. My own studies, as well as those of Hudack and McMaster, and the more recent work of Lurie, indicate that there seems to be perhaps an increased, or at least an unimpeded lymph drainage in the very *initial* stage of an acute inflammation. I believe that, just as there is an increase in capillary permeability at the beginning of inflammation, there is likewise an increase in the permeability of the lymphatics. However, as the inflammatory reaction progresses in intensity, as evidenced by a comparison of observations on the fixation in the inflamed area with data obtained on the accumulation of substances from the blood stream, lymphatic blockage evidently seems to occur earlier, or at least more effectively, than the subsequent thrombosis of vascular capillaries. This may suggest that the afferent lymphatic vessel is more delicate in structure than the capillary wall and consequently more susceptible to injury.

The time relation is of the utmost importance in properly evaluating the fixation capacity of various microörganisms. An understanding of this time factor would aid in the elucidation of the rôle of inflammation in regulating the invasiveness of various pyogenic microörganisms. It might explain the differences in the extent of the immediate spread of various types of bacteria from the site of their inoculation and the significance of these variations. This point can be illustrated by a diagram:



Time: Duration of local inflammation after inoculation with pyogenic bacteria.

The abscissa represent in a diagrammatic manner the duration of the inflammatory reaction from the time of cutaneous inoculation of different pyogenic organisms. The ordinate on the left (reading downward) indicates the intensity of local fixation as determined by lymphatic blockade. The ordinate on the right (reading upward) indicates the dissemination or spread of bacteria from their point of inoculation, as measured by their recovery in the regional lymphatics or in the general circulation. Three pyogenic organisms have been studied in regard to their invasiveness: *Staphylococcus aureus*, *Pneumococcus* Type I, and *Streptococcus hemolyticus*. The *Staphylococcus aureus*, probably owing to its highly necrotizing action, induces prompt walling-off of the inoculated area. Lymphatic blockade occurs early, *i.e.* a little over 1 hour after bacterial inoculation, as indicated on the diagram by the intersection point B. There is nothing to prevent the spread through lymphatics of the *Staphylococcus aureus* between the time of inoculation (*A*) and the time of fixation (*B*). In the case of *Pneumococcus* Type I, fixation occurs somewhat later, as indicated by the intersection point (*C*). Hemolytic streptococci are capable of disseminating through patent lymphatics for almost 2 days before the local reaction is sufficiently intense to prevent their free spread (see point *D* on the graph). It is clear that we are dealing with an important time factor in regard to the effectiveness of fixation by a given microörganism. This factor should doubtless be taken into account in connection with invasiveness. The fixation of a microörganism is a function of its capacity to induce local injury. The *Staphylococcus aureus* organisms which can reach regional lymphatic nodes in the interval between (*A*) and (*B*) are probably taken care of either by phagocytosis in these structures or perhaps by bactericidal substances in lymph or blood. The same holds for pneumococci or streptococci spreading in the interval between *A* and *C* or *A* and *D* respectively. The difference in the number of disseminating microörganisms is thus a quantitative expression depending on the intensity of the local response. The virulence of a particular microörganism may be so elevated that the bacteria reaching the circulating blood stream in the interval prior to the development of fixation may be sufficient in number to overcome the resistance of the host.

In immunized animals the rapidity of fixation is doubtless accelerated and reinforced by the presence of antibodies. I am fully aware of Freund's work in which antibodies were shown to be present in the tissues of an immune animal. Nevertheless, I have demonstrated that foreign proteins readily concentrate from the circulation in an inflamed area. This is in part due to the increased capillary permeability which may occur as early as 2 minutes after the injection of an irritant and is demonstrable by methods other than microscopic observation. Consequently the almost immediate increase in the local concentration of immune antibodies which probably occurs in a reinoculated hypersensitive animal may be of significance in delaying the spread of the reinfecting homologous bacteria. Therefore my question is — should not one consider the initial retarding reaction to the spread of reinfecting bacteria as at least in part an initial phase of the specific inflammatory process? The augmentation in capillary permeability must undoubtedly occur upon reinfecting an immune animal. For this reason in a rabbit immunized, for instance, to *Pneumococcus* Type I the time and intensity of fixation would be accelerated partly by the early concentration of antibodies and the point of intersection on the graph would be shifted to the left at *C'*. We still need much more information on some of these problems, including the exact rôle of the allergic inflammation in its relation to immunity, a subject, however, which time does not permit me to discuss at present.

(Dr. Rich, closing.) In reply to Dr. Longcope's question as to why the lesions of the second infection are smaller, there is no question but that the fever temperature of the animal plays a very great rôle. We think that the reason the second lesions are larger in most animals reinfected in the earlier stages of the first infection than in those infected after 24 hours is because it takes time for the temperature to rise, and the fever response is inconstant in the early stages of the infection. Briefly, if the rabbit's temperature is raised by placing the animal in a hot box before the bacteria are injected, and if the animal is kept in the hot box during the experiment, the size of the lesion will be restricted as much as by a previous infection.

I am sorry, Dr. Moon, that I cannot throw any worthwhile light on the question of the mechanism of inflammatory hyperemia or chemotaxis. I am glad that Dr. Dixon and Dr. McCutcheon will report studies bearing upon the latter problem this afternoon.

With regard to Dr. Sulzberger's remarks, I did not mean to imply that hypersensitivity is beneficial in infections. What I said was that it is probable that the potentiality to develop the state of hypersensitivity serves a useful purpose in some direction, even though it may be acting out of place in bacterial infection. What useful function it serves, however, I do not know.

In regard to Dr. Freund's question as to whether the rapidly acquired resistance of the rabbit to the Type III *Pneumococcus* is a specific immunity reaction, so far we have no evidence that it is. It seemed hardly credible that so high a degree of specific immunity could be developed in such a short time and, as I have said, the fever response is certainly a most potent non-specific factor in resistance to this bacterium. It is of course known that antibodies may develop with surprising rapidity after the inoculation of large amounts of pneumococcal vaccine, but we have not yet been able to demonstrate any specific factor by passive protection experiments in the present case.

As to the function of the eosinophiles, I know nothing of importance about that. We all know that these cells are commonly increased in association with animal parasites and some other foreign proteins, but the meaning of the reaction is obscure. The work of Campbell indicated that their appearance was conditioned by the state of hypersensitivity, but this is not yet definitely established. Even if this were true for foreign animal protein, hypersensitivity to bacterial protein rarely entails an eosinophilic response.

In regard to Dr. Mudd's question, whether there may be a stage in the development of immunity, before antibody is well developed, in which hypersensitivity may be the mechanism of protection, we have not been able to find such a stage, if it exists, and we have sought pointedly to find it. It is quite true that there are immune animals in which we cannot demonstrate serum agglutinin or precipitin by the ordinary *in vitro* tests, but I am not sure that that means there is no protective antibody present in the body of those animals. That anaphylaxis is dependent on antibody is clear, but a perfused uterus still reacts anaphylactically even though no antibody can be demonstrated in the perfusate. It is not difficult for me to believe that an effective amount of protective antibody may be present in the body and yet not be present in sufficient amount in the serum to be recognized by our present tests. At all events, we have never encountered an animal, whether in an early or a late stage of immunization, that did not remain immune following the abolition of hypersensitivity.

In regard to Dr. Dienes' conclusion that immunity in pneumonia is dependent on factors other than antibody and phagocytosis for the reason that if you inject

antibody on the 3rd or 4th day it does not cure the disease so effectively, there are several very important points to be considered there. In the first place, it is a question whether the antibody escapes into the alveoli and reaches the bacteria when it is injected late or not. You will remember Winternitz' work, showing that if pneumonia is produced experimentally and a colloidal dye injected intravenously at different periods after the introduction of the bacteria into the lung, the dye will escape into the alveoli only in the early stages of the inflammation. In later stages the dye will not penetrate into the consolidated area, and the exudate consequently remains unstained. It is therefore a question whether the antibody escapes into the alveoli freely when injected late. Furthermore, it is important to know whether or not the leukocytes of the exudate are in a healthy condition and able to phagocytoze efficiently after having been exposed to the proliferating pneumococci out in the alveoli for a number of days. Another point to be considered is the fact that with the passing of every day the number of bacteria to be combated increases enormously in the non-immune body. Finally, there is the possible effect of an accumulation of soluble specific substance in the exudate which may neutralize antibody if it escapes into the alveoli. We must at least know the answer to these and other similar questions before we depreciate too readily the rôle of antibody and leukocytes in recovery from pneumonia. In view of our present information regarding the remarkable efficacy of the antibody-leukocyte mechanism in protection against the pneumococcus, I certainly tend to believe that if the injected antibody reached the alveoli and covered the bacteria, and if the leukocytes were alive and not damaged, late serum treatment would be far more hopeful. At all events, I do not think that the failure of late serum treatment is as simple an argument against the importance of antibody as Dr. Dienes' remarks might indicate.

I quite agree with Dr. Dienes that much experimental work on hypersensitivity has been done with too large doses of antigen, although we must remember that not infrequently large numbers of bacteria do produce large hypersensitive lesions in the human being — in tuberculosis, for example. However, the assumption that hypersensitivity is necessary for protection in lesions in which only a few bacteria lodge at a site is one which at least requires proof. We have investigated this matter, using very small as well as large doses of bacteria, but whether large or small doses were injected we have never been able to find an animal in any degree or stage of immunity in which, if we got rid of the hypersensitivity, immunity would not act as efficiently in the localization and destruction of the bacteria. All that I should like to say in regard to this question is what I said in my paper: Let us not make any assumptions one way or the other, but if there is any particular situation in which one feels that hypersensitivity is beneficial, abolish the hypersensitivity, introduce the same number of bacteria, and see whether or not the animal survives in the same way. I am just as eager as Dr. Dienes to find a use for hypersensitivity, but so far I have not been able to do so.

Finally, I agree with Dr. Menkin that there is no conflict between our views regarding the fact that fibrin assists in preventing the spread of bacteria. I would, however, question whether inflammatory fixation is accomplished chiefly by a mechanical fibrin obstruction, or whether physicochemical factors, promoting the adherence of the bacteria to the fibrin strands and to the tissues, may not play an important rôle.

STUDIES ON THE FATE OF LIVING BACTERIA INTRODUCED INTO THE UPPER RESPIRATORY TRACT OF NORMAL AND OF INTRANASALLY IMMUNIZED RABBITS. Paul R. Cannon and (by invitation) Theodore E. Walsh, Chicago, Ill.

Abstract. These studies were undertaken for the purpose of determining (1) to what extent living bacteria can penetrate the mucous membranes of the upper respiratory passages of normal rabbits, (2) by what routes bacteria in the upper respiratory tracts may reach the lungs and the general blood stream, and (3) the effects of local immunization of the upper respiratory passages on these processes.

Normal rabbits were used in the first part of the study as follows: Broth cultures of staphylococci or Type I pneumococci were instilled into the nostrils, and at intervals portions of the lungs, liver and spleen were removed aseptically and the germ-content per 0.1 gm. of tissue determined. Blood cultures were also made and portions of the upper respiratory tract studied histologically. Rabbits were also immunized intranasally by the instillation, usually for 5 days, of a formalin-killed culture of Type I pneumococci, after which living pneumococci were instilled into the nostrils and the fate of the pneumococci determined as described for the normal animals.

The conclusions may be summarized as follows: Living bacteria suspended in fluid and introduced into the nostrils do not penetrate the mucous membranes of the upper respiratory tract either quickly or readily, regardless of whether the bacteria are virulent or non-virulent. The mucous membranes constitute an effective barrier; the bacteria tend to be caught in the film of mucus and may remain in the nasal passages for considerable periods of time. Many of the bacteria, however, pass quickly from the nose to the lungs, both of normal and of intranasally immunized rabbits, reaching the lungs by direct aspiration, as shown by the fact that large numbers of microörganisms may be present in the lungs at a time when the blood, liver and spleen are sterile. Virulent pneumococci in the lungs pass into the blood and eventually cause septicemia and death in normal rabbits: this process is less active in intranasally immunized animals and such animals can withstand large infecting doses intranasally. Intranasal immunization of rabbits, therefore, provides an effective and simple method whereby both the lower respiratory tract and the body as a whole are protected from infection of the upper respiratory tract.

Discussion

(Dr. Herbert S. Reichle, Cleveland.) I should like to ask Dr. Cannon from which portion of the lungs he took his representative sections.

(Dr. Alwin M. Pappenheimer, New York City.) I think Dr. Cannon's observations will probably apply to human lungs also. A good many years ago Dr. Norris and I tried some experiments of instilling test organisms, like *B. prodigiosus*, into the mouth and nostrils of moribund patients, and even after death, and we were able to recover them from the lungs in a large proportion of the cases. Probably in humans also there is a very ready passage.

(Dr. Stuart Mudd, Philadelphia.) Dr. Cannon, can you get similar effects by immunizing in the ordinary way?

(Dr. Cannon, closing.) In reply to Dr. Reichle's question, we ordinarily have taken the tissues from the lower lobes, but at times we have also taken them from the upper lobes. There seems to be a variation on the part of the lung in

which this particulate material arrives, and some of these variabilities may be explained on that basis.

In regard to Dr. Pappenheimer's observation on the human being, we have wondered about that, and had thought of trying the same thing some time in a moribund patient. We have observed that if 1 cc. of a bacterial suspension is put into the mouth of a normal rabbit, large numbers of the microörganisms may be found in the lungs 30 minutes later. We have been interested particularly in the application of these findings to the pathogenesis of pneumonias of children, and especially to the lipoid pneumonias.

We have not studied the effects of general immunization in any great detail as we were simply trying to determine what influence local immunization may have on the processes under consideration. I do not know how one could immunize animals generally and locally and have the two sets of conditions strictly comparable. I presume, however, that general immunization would have a similar effect so far as the reactivity of the liver and spleen is concerned, but I do not know how it would influence the ability of the lungs to localize and destroy bacteria entering by way of the bronchi. We do not wish to imply that local immunization is a thing apart from general immunization: it is difficult to discuss the former type of procedure and at the same time keep from giving the impression that it is separate and distinct from the latter, but we do not intend to make any sharp distinction other than to suggest the possibility that direct antigenic application to an important portal of entry may accomplish an immunizing effect simply and perhaps adequately.

THE PASSAGE OF NON-SPORULATING ANEROBIC BACTERIA FROM THE LUNGS INTO THE BLOOD STREAM. Charles Weiss, San Francisco, Calif.

Abstract. It has recently been suggested by Tuttle and Cannon, and Neufeld and Kuhn that the passage of certain aerobic bacteria from the lungs into the blood stream might be of use as evidence of their invasiveness. The last two authors, however, demonstrated that bacterial toxins may produce an increased permeability of the alveoli, thus facilitating the penetration of pneumococci into the circulation. Since no conclusive evidence has yet been brought forth concerning the pathogenicity of several anerobic, non-sporulating bacteria (including *B. melaninogenicum*, anerobic streptococci and anerobic Vibrios) which are associated with chronic pulmonary abscess and oral infections, this method of investigation was therefore applied to them.

Pure cultures of several of these organisms singly or in various combinations were injected intrabronchially into the lower lobe of the lungs of Java monkeys (*Macacus irus*), under fluoroscopic control, by means of a radiopaque X-ray catheter. The expulsion of the inoculum was prevented by the introduction through this catheter of a "plug" of 1.5 cc. of melted agar. Blood cultures were taken from the femoral vein at intervals of 10 to 15 minutes and at 24 and 48 hours after injection, as well as after the death of the animal or at autopsy.

When pure cultures of an anerobic streptococcus or an anerobic, Gram-negative, non-motile bacillus, or a combination of these with fusiform bacilli, *B. melaninogenicum*, and a Vibrio-like Gram-negative motile rod were injected into normal, uninjured lungs of monkeys, the blood cultures usually remained sterile. Occasionally there was a transitory bacteremia due to the presence of the Gram-negative, non-motile rod, but this organism disappeared within 24 hours. When a pathogenic strain of *Staphylococcus aureus* was combined with

several of the above anerobes and injected into a normal lung, only the staphyloccus made its appearance in the blood stream.

In another series of animals the lungs were first injured by the intrabronchial injection of the toxins of *Staphylococcus aureus*, and the various anerobic organisms referred to above were then injected intrabronchially 48 hours later. It was observed that these bacteria, as well as some which are normally present in the animal's deeper respiratory passages, penetrated into the blood stream and persisted there for 24 to 48 hours. In some instances the anerobes attacked the injured pulmonary tissue and produced necrotic abscesses involving practically the entire lobe of the lung.

It is tentatively concluded on the basis of these and other data, that cultures of fusiform bacilli, *B. melaninogenicum*, anerobic streptococci and the so-called Vibrios, which were isolated from cases of lung abscess and maintained on laboratory media for a short time, are not capable of injuring the normal monkey lung or of passing through it into the blood stream. They may, however, invade and destroy tissues which have been previously injured by the toxins of other bacteria.

THE RÔLE OF THE ANEROBIC STREPTOCOCCUS IN INFLAMMATION. J. G. Henthorne and J. R. McDonald (by invitation), Rochester, Minn.

Abstract. Described by Veillon in 1893, anerobic streptococci have been studied by Prévot, Schottmüller, Brown and others.

Previous to April, 1935, one of us recovered anerobic streptococci in cultures in 12 cases in which death was caused by gangrenous suppuration. In 1 case the organisms were in pure culture; in the remainder, the flora was mixed. Since April, 1935, eleven strains have been maintained for bacteriological investigation.

The anerobic streptococcus lacks pathogenic properties for laboratory animals when ordinary methods of inoculation are employed. However, meningitis, ophthalmitis, and orchitis have been produced in rabbits, although the latter two types of lesions had a marked tendency to undergo spontaneous resolution. The anerobic streptococcus proved virulent for all laboratory animals only when introduced in symbiosis with a strain of another genus of bacteria.

From biological reactions of the anerobic streptococci we have been able to classify the eleven strains into eight different species.

Discussion

(Dr. Charles Weiss, San Francisco.) The anterior chamber of the rabbit's eye is not suitable for the study of pathogenicity of microörganisms since so-called saprophytes like *B. subtilis* or *B. prodigiosus* when thus injected will produce a severe panophthalmitis.

THE RELATION OF STREPTOCOCCI TO THE VIRUSES OF POLIOMYELITIS AND ENCEPHALITIS. Edward C. Rosenow, Rochester, Minn.

Abstract. By the use of special methods, especially of cultivating streptococci in a new medium, autoclaved chick-mash,* it has been possible to produce from streptococci far removed from their original source, transmissible, filtrable viruses of encephalitis and poliomyelitis. This has been done with streptococci

* Rosenow, E. C. *Proc. Staff Meet. Mayo Clinic*, 1935, 10, 410–414.

isolated from the nasopharynx and spinal fluid during life, and from the brain and spinal cord of persons who have succumbed to encephalitis or poliomyelitis; it has also been done with streptococci from "natural" passage viruses and from milk and water supplies during epidemics of these diseases, and with certain strains of streptococci from sources remote from encephalitis or poliomyelitis. Neurotropic cataphoretic velocity and virulence of the streptococci appear to be essential for the production of the "virus" phase.

The symptoms and lesions in animals, especially monkeys, while variable at first, became more typical with successive passage. In the early "virus takes" the streptococcus could be re-isolated quite readily, but this became increasingly difficult with successive passages through animals; the streptococcus appeared and disappeared in successive cycles of from 3 to 5 or more passages. *Macacus rhesus* monkeys which had recovered with residual paralysis following inoculation of "natural" poliomyelitic virus proved resistant to highly virulent, experimentally produced virus. "Takes" at crucial points occurred simultaneously in more than one of a small series of animals inoculated and in two or three different species. Inoculated control animals never yielded transmissible virus or revealed lesions of encephalitis or poliomyelitis

ANTIBODY IN RELATION TO IMMUNITY TO ACUTE POLIOMYELITIS. John A. Kolmer, with the assistance of Anna M. Rule, Philadelphia, Pa.

Abstract. The antiviral substance occurring in the blood appears to be a true antibody capable of neutralizing but not necessarily destroying the virus of poliomyelitis.

This antibody occurring in the blood of normal human beings appears to be identical in its properties with that produced by infection and vaccination of monkeys and human beings.

The origin of the natural antibody is uncertain but it may be the result of unrecognized infection, antigenic stimulation by common substances or haptens occurring in various bacteria and other substances, or the product of normal growth.

The prophylactic value of this antibody in normal and immune serums for human beings is uncertain but probably slight and of short duration, due in part to inadequate dosage.

There is no irrefutable statistical proof of the efficacy of the antibody in the treatment of poliomyelitis of human beings nor any absolute proof of its complete ineffectiveness. It is possible and probable, however, that *large* amounts administered *early* in the disease may effectually prevent progressive infection of the spinal cord by the neutralization of virus.

The antibody, however, has demonstrated some protective activity in monkeys inoculated intracerebrally with virus and especially when administered in large amounts per body weight early in the incubation period.

The immunity produced in monkeys and human beings by infection or immunization with living virus (active, glycerinated and ricinoleated) appears to be largely cellular (tissue) but may be partly humoral due to antibody.

Over 90 per cent of monkeys immunized with living virus by various investigators showed the presence of antibody in the blood on the basis of serum neutralization tests. Practically 100 per cent injected subcutaneously or intracutaneously with 5 doses of ricinoleated virus in amounts of 0.1 to 0.5 cc. per kg. showed the presence of the antibody in the blood. In our laboratory the majority

of these monkeys were completely protected against the intracerebral injection of virulent virus and we believe that the resistance, while probably largely cellular, may be due in part at least to the antibody in the blood.

PHYSIOLOGICAL OR STRUCTURAL HOST FACTORS IN THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM INFLUENCING THE INVASIVENESS OF CERTAIN NEUROTROPIC VIRUSES AND THEIR POSSIBLE RÔLE IN THE EPIDEMIOLOGY OF POLIOMYELITIS. Albert B. Sabin (by invitation) and Peter K. Olitsky, New York City.

Abstract. Since the *rhesus* monkey, as was recently emphasized by Flexner, apparently does not respond to the virus of poliomyelitis in precisely the same manner as man, an attempt was made to discover, among other experimental virus diseases affecting chiefly the nervous system, a "model" for some of the unexplained facts in the epidemiology of poliomyelitis.

Studies on the virus of vesicular stomatitis in mice and guinea pigs have led to the conception that the progression of a neurotropic virus along the nerves from the periphery to the central nervous system (CNS) may be arrested at various sites as a result of certain physiological or structural host factors. These host factors vary with age and with the species of animal infected. Thus in young mice, virus introduced in the nose progresses along the olfactory nerve to the olfactory region of the brain, and from there to the thalamus, cortex, cerebellum, midbrain, and so on, giving rise to a fatal encephalomyelitis, while in older mice the virus reaches the olfactory region of the brain by the same route but stops there, not because of the insusceptibility of the remainder of the CNS but as a result of some local block; the older animals remain well and do not show any clinical signs of disease (see *Am. J. Path.*, 1935, **11**, 839). In this instance, the block is central and develops with age in all but a few animals. When the virus is inoculated by other peripheral routes in mice there is also a block which develops with age but it appears to be peripheral. For example, after injection of the virus into the leg muscles of young mice it ascends along the sciatic nerve, enters the spinal cord, and thence upward to the rest of the CNS, giving rise to paralysis and ultimately death, while in older mice it enters neither the nerve nor the CNS and the animals remain well. Since inoculation of the virus directly into the sciatic nerve of older mice results in its further progression and development of paralysis and death as in the young, it appears that here the barrier is chiefly in some structure between the nerve and the muscle. After intraocular inoculation the virus is found to invade by way of the optic and fifth nerves in young mice, and again its invasion is arrested peripherally rather than centrally in older animals.

Variations of behavior of neurotropic viruses in different species may be considered on the basis of similar local blocks in the peripheral and central nervous system. While the CNS of young guinea pigs and mice is equally susceptible to the virus, peripheral inoculation rarely induces encephalomyelitis in the former, but does so uniformly in the young mice. Virus introduced in the nose of young guinea pigs appears to be arrested at the thalamic region and does not reach the cerebellum, midbrain, medulla and spinal cord; in older guinea pigs, however, the block is peripheral, for the virus does not reach even the olfactory bulbs. After injection into the leg muscles, the block is again peripheral, *i.e.* somewhere between the muscle and nerve, in both young and old guinea pigs, for inoculation

directly into the sciatic nerve results in the spread of the virus to the spinal cord and the remainder of the CNS, inducing paralysis and ultimately death.

Mice and guinea pigs, in which invasion of virus is sufficiently arrested to prevent clinical manifestations of disease, nevertheless develop specific antiviral bodies.

Certain analogies are clearly discernible between the manifestations of the experimental disease just described and certain phenomena encountered in the epidemiology of poliomyelitis. The possible rôle of structural or physiological host factors in the peripheral and central nervous system merits consideration as a hypothesis for the explanation of these phenomena.

Discussion of papers by Drs. Kolmer, Sabin and Olitsky

(Dr. Stuart Mudd, Philadelphia.) I presume there was no possibility that the adult animals could have had previous contact with the virus?

(Dr. Albert Sabin, New York City.) No. The possibility of spontaneous infection with this virus was excluded by the fact that the mice were carefully bred and kept under isolated conditions until used, that all were equally susceptible to infection by direct intracerebral inoculation, and by the demonstration that resistant animals contained no antibodies in their blood before exposure to the virus.

I should like to discuss Dr. Kolmer's paper. Dr. Kolmer's conclusions, if I understand them correctly, are (1) that the resistance of monkeys to poliomyelitis bears a definite relation to the content of antiviral body in their serum, (2) that the antibody content is greater in the serum of convalescent monkeys than in that of vaccinated monkeys, and (3) that the failure of Olitsky and Cox to induce an active resistance to infection with poliomyelitis in most monkeys treated with ricinoleated vaccine may be attributed to the fact that insufficient virus was administered. I regret to say that further studies on the same subject by Dr. Olitsky and myself have led us to adopt essentially opposite conclusions. Olitsky and Cox are not alone in maintaining that vaccinated monkeys may frequently exhibit appreciable antibody in the blood without being resistant to infection by way of the nose: Schultz and Gebhardt, Aycock, Hudson and coworkers have all obtained similar results using varying amounts of virus, both larger and smaller than that used by Dr. Kolmer. In a recent investigation, Dr. Olitsky and I undertook to determine in what way vaccinated and convalescent monkeys differed in resistance to infection by the nasal route and in the content of antibody in their blood. Convalescent monkeys were found to be resistant to reinfection with the same strain of virus when tested at intervals of 3 weeks to 3 months after the onset of paralysis. Antibody formation in them was very much slower, however. In none of the convalescent monkeys tested 1 month after onset of paralysis was there demonstrable antibody in the blood; some of them showed antibodies for the first time at 2 months, while others not until 3 months. We thus had convalescent monkeys without demonstrable antibody which were resistant to infection, and vaccinated monkeys with antibody which were not resistant. Titrations on the serums of convalescent monkeys which finally developed antibody indicated that quantitatively there was no appreciable difference between them and the serums of non-resistant vaccinated monkeys. Our conclusion for the present, therefore, is that the relation between antibodies in the blood and resistance of monkeys to infection with poliomyelitis is obscure, if

one actually exists, and that convalescents possess some mechanism of resistance which many vaccinated monkeys apparently lack.

(Dr. Kolmer, closing.) As stated in my paper, I believe it is very difficult to arrive at any definite conclusion on the rôle that antibody may play in resistance and immunity to poliomyelitis. But, while the protective or prophylactic activity of antibody in normal and immune serum has been of doubtful value in human beings, yet from the experiments of others and ourselves it would appear that large doses are capable of protecting a percentage of monkeys when given within a day or two after intracerebral or intranasal inoculation with virus.

Monkeys immunized with adequate amounts of vaccine carrying active virus almost always show the presence of antibody in the blood and the majority have proved resistant to intracerebral inoculation of virus. With my ricinoleated vaccine 5 doses of 0.1 cc. per kg. are not always sufficient and, unfortunately, investigators have not tested it for the presence of active virus by injecting 0.3 cc. intracerebrally in the monkey, as has been our custom. We never use a vaccine unless this preliminary test shows the presence of sufficient active virus to produce paralysis within 14 days after inoculation, as vaccines without this amount of virus have failed to immunize monkeys. We have not generally employed intranasal inoculation as a test for acquired immunity because of irregularity in infection of the controls. Whether or not the acquired immunity is due to humoral antibody or tissue resistance, I am not prepared to state. I believe, however, that active immunization of monkeys with my vaccine induces an important tissue resistance, but it is likely that the antiviral antibody in the blood may also play some part in the immunity.

A Classification of Primary Inflammation of Arteries. Howard T. Karsner, Cleveland, Ohio.

Abstract. The extension of inflammation so as to involve arteries is well known, as is also inflammation of arteries due to the direct invasion of parasitic agents. These inflammations are obviously secondary in character. There is a group of arterial inflammations which cannot be included in this category. In order to distinguish this group the term "primary" is suggested. In order to forward the study of these lesions a classification is proposed as follows:

ARTERITIS

Acute Alterative (degenerative)
Necrotizing
Exudative
Vegetative (thrombo-arteritis)
Proliferative
Organizing

Chronic — combinations of
Intimal
Medial
Adventitial

The alterative or degenerative form of acute arteritis is observed in many infectious diseases, independently of direct bacterial invasion. Swelling and deterioration of muscularis and elastica are evident, often associated with edema, but without other signs of inflammation. That they are inflammatory is indi-

cated by the later appearance of definite exudation. The same is true of some of the necrotizing forms. The other terms are self explanatory.

Chronic arteritis differs from arteriosclerosis in distribution of fibrosis in the various coats and by delayed or absent secondary changes in the fibrous tissue.

Discussion

(Dr. Paul Klemperer, New York City.) Dr. Karsner's classification is extremely interesting to me, and I think it will help to clarify the point of view of authors describing the histological lesions encountered. However, the difficulty, it seems to me, is not altogether removed in differentiating between the so-called degenerative and inflammatory lesions of arteries, particularly in chronic arterial lesions. I should like to ask Dr. Karsner how he would classify the lesions in the interlobular arteries in malignant nephrosclerosis. Does he consider them as inflammatory lesions, or closer to arteriosclerosis? This brings up again the old question as to whether one should regard arteriosclerosis as a degenerative or as a chronic inflammatory lesion.

(Dr. Alfred Plaut, New York City.) I should like to inject one point into this discussion of primary inflammatory arterial lesions, and that is the size of the arteries involved. One can see from Dr. Karsner's photomicrographs that in his material his attention was mostly directed to the smaller arteries, and there are occasional, and perhaps even frequent, instances where one sees inflammatory processes restricted to a certain size of artery or arteriole. In septic cases, if I may use that term, one sometimes finds precapillary arterioles, notably in the kidney pelvis and in the testicle, with necrotic lesions such as Dr. Karsner has shown. In very rare instances of arteritis in the lung, only vessels of a certain caliber are affected. In the vermiform appendix and occasionally in the ovaries, the precapillary arterioles sometimes are the seat of a chronic infectious process. We may, in future, have to make further subdivisions of the term artery, not only anatomically, but biologically, also. We probably will find that certain pathological processes, and especially certain immunological reactions, take place in arterial vessels of a definite caliber.

(Dr. Karsner, closing.) In reply to Dr. Klemperer, attention is drawn to the fact that this is a study of arteries rather than of arterioles. Technically, the examination of arteries is more readily made than is the case with arterioles. Thus in the study of the latter, matters of opinion are more likely to appear. I have the deepest respect for Dr. Klemperer's studies and opinion in regard to arteriolar disease. My own opinion is that the common forms of arteriolar disease, whether they be a malignant disease in young individuals, a superimposition of degenerative or exudative lesions on chronic arteriolar disease, or chronic disease itself, are forms of inflammation in the broad sense.

It is gratifying to have Dr. Plaut emphasize the curious manner in which disease of arteries is often limited to vessels within a certain range of size, may sometimes be generalized and may sometimes affect only one of a limited number of organs and regions. I am grateful for the criticisms of both Dr. Klemperer and Dr. Plaut.

(Dr. S. Burt Wolbach, Boston.) I deprecate the use of the words inflammatory and degenerative when specific connotations are implied in regard to lesions of unknown pathogenesis. Inflammatory reactions are often, possibly always, responses to extraneous factors brought into direct contact with tissues. The use of "degenerative," I think, should be employed to express changes in tissues

occasioned by factors which are injurious only through their effects on tissue metabolism.

Degenerative lesions, because of the mechanical weakness they occasion, may be followed by cellular and exudative responses commonly regarded as inflammatory. The histological sequences in experimental scurvy best illustrate this. I make a plea for great caution in the use of "degenerative" and "inflammatory" where etiological connotations are implied.

(Dr. Karsner, closing.) The nature of processes and conditions is of necessity subject to definition. Definitions are not mere collections of words but are orderly arrangements of words outlining or clarifying a concept. In the case of inflammation, the definitions, as is well known, vary greatly as to scope, and I choose to select the broadest of these definitions.

EXPERIMENTAL NEPHRITIS IN RATS INDUCED BY ANTIKIDNEY SERUM. Joseph E. Smadel (by invitation), New York City.

Abstract. Antiserums that are toxic for rats can be prepared in rabbits by injecting emulsions of perfused rat kidney. Such serums contain several factors; one of these affects the kidney primarily but is not strictly organ specific. This factor is absorbed by homologous kidney and liver cells and by fat-free kidney suspensions, but not by petrol-ether-soluble kidney lipoids, or by red blood cells or serum. It is found in the globulin fraction and occurs in varying amounts in some serums prepared by immunizing rabbits against other organs.

The nephrotoxic factor induces a disease characterized by albuminuria, cylindruria, anasarca, and elevated blood urea, without significant hematuria. The outstanding histopathological changes in the acute stage are tubular degeneration and thickening of the glomerular capillary basement membranes. Some rats recover to a greater or lesser degree. Chronic progressively affected animals, followed up to 10 months, show extensive tubular change—interstitial scarring, and thickened capillary basement membranes, crescent formation and glomerular scarring with various stages coexistent in the same area.

The proper amount of antikidney serum induced a less specific process characterized by the additional features of hematuria, death in 1 to 8 days and extensive fibrin thrombosis of glomerular capillaries. This apparently depends on the action of nephrotoxin in conjunction with other factors.

Discussion

(Dr. David P. Seecof, Montreal.) I should like to ask whether controls were available of animals kept under similar conditions, say for 200 days. In a colony of rats that have been inbred for a long time it is not rare to find such renal lesions. These animals never received nephrotoxin.

(Dr. James P. O'Hare, Boston.) Were there any acute changes outside the kidney comparable to those in the kidney? Chronic changes you showed, but I wonder if there were acute changes as well.

(Dr. Smadel, closing.) The acute phase of nephrotoxic nephritis can be induced without accompanying significant lesions in organs other than the kidney; the anaphylactoid reaction must be avoided, however. When the anaphylactic-like response is severe enough to result in hematuria and fibrin thrombi in the glomerular capillaries then other organs are affected, and a general vascular phenomenon with hemorrhage and transudation is observed.

Between 350 and 400 rats were used in these experiments: slightly over half received nephrotoxic serums; the remainder received other antiserums or were uninoculated. Approximately 50 of this latter group were observed for from 6 months to a year; 20 of these, uninoculated controls, were kept on a diet of only bread and milk. Some of the animals on this poor diet developed albuminuria and hematuria and were found to have pyelonephritis; *B. enteritidis* was isolated from the kidney. Chronic lesions comparable to those developing after nephrotoxin were not observed in control animals.

THE INADEQUACY OF ALLERGIC INFLAMMATION IN PROTECTION AGAINST INFECTION WITH VIRULENT PNEUMOCOCCI. Paul R. Cannon and (by invitation) George Hartley, Jr., Chicago, Ill.

Abstract. These studies were undertaken in an attempt to determine to what extent allergic inflammation may modify infection with a virulent pneumococcus. Rabbits were made allergic by repeated subcutaneous injections of egg albumin until they gave positive Arthus reactions. Other animals were actively immunized by the subcutaneous injection of formalin-killed pneumococci. Later, these animals and normal controls were injected subcutaneously with graded dilutions of living Type I pneumococci suspended in a solution of egg albumin. If allergic inflammation occurs quickly and effectively, it should alter the ability of the pneumococci to escape from the site of infection, and if adequate, should protect the animals when comparatively few bacteria are injected.

The results showed no protection whatever. Allergic animals, when injected with only 50 living pneumococci, died as quickly as the normal controls. Even when the allergic inflammation was present for 2 to 3 hours before injection of the pneumococci into the site of inflammation, the animals died as promptly as did the controls. Immune animals, on the other hand, survived much larger doses of infecting organisms, demonstrating the superiority of immunity over allergic inflammation, when dealing with highly virulent microörganisms.

Discussion

(Dr. George Packer Berry, Rochester, N. Y.) I wish to report a situation with a filterable virus analogous to that just described by Dr. Cannon. For a number of years we have been studying the relation of the viruses of rabbit fibroma (Shope) and infectious myxomatosis (Sanarelli). Recently we described a method for changing fibroma virus, which produces a benign type of local infection, into myxoma virus, and which always leads to a generalized reaction which invariably kills domestic rabbits. In attempting transformation experiments in the reverse direction, *i.e.* from myxoma virus to fibroma virus, we have attempted many procedures designed to localize myxoma virus to the site of inoculation. With this objective we have tried to fix myxoma virus in areas of allergic inflammation, induced in different ways by a variety of suitable proteins. All our attempts failed. As in Dr. Cannon's experiments, allergic inflammation was inadequate in preventing generalization of the infectious agent.

(Dr. Esmond R. Long, Philadelphia.) In connection with the experiments which Dr. Cannon has reported, and also those to which Dr. Rich referred this morning, I wish to comment on an experiment which my colleague, Dr. Lurie, has performed. Dr. Rich pointed out that it has always seemed plausible that the overwhelming inflammation in the allergic reaction would be the factor re-

sponsible for the rapid destruction of bacteria. Contrary to the original experiments on the rapid localization of bacteria in allergic inflammation, Dr. Lurie had this experience. If he injected similar quantities of tubercle bacilli into the legs of normal and tuberculous rabbits and guinea pigs and counted the number of bacteria found 24 hours later in the regional lymph nodes, he discovered, contrary to what might be expected on the basis of rapid localization, that there were more tubercle bacilli in the animals that gave the allergic reaction than in the others. If the tubercle bacilli were mixed with Trypan blue or India ink before injection, the color of the lymph nodes gave similar evidence of more rapid drainage in the allergic animals. Subsequent to the acute response the bacilli were destroyed more rapidly in the allergic animal, both in the lymph nodes and at the site of injection. If he mixed tubercle bacilli with agar and injected them into animals, so that they were held mechanically, in those animals with allergic inflammation a rather rapid concentration and destruction of the tubercle bacilli took place, in spite of the fact that cells of inflammation could not reach many of them. This is in agreement with Dr. Cannon's experiments, and indicates that there is something outside of the cells, perhaps produced from them which is responsible for the retardation of growth of tubercle bacilli, and ultimately destroys them. This changes our conception of the way in which the allergic reaction is responsible for the localization of bacilli.

THE PHAGOCYTIC ACTIVITY OF CIRCULATING CELLS IN LEUKEMIAS. Max M. Strumia, Bryn Mawr, Pa.

Abstract. Experiments on phagocytosis, employing leukocytes circulating in normal subjects as well as all forms of acute and chronic leukemias, and in the so-called glandular fever (acute mononucleosis) gave the following results:

Normal Blood: Maximal phagocytic activity is displayed by polymorphonuclear neutrophiles followed closely by monocytes. Eosinophiles also have distinct phagocytic activity, but less than that of previous groups. Lymphocytic cells display no phagocytic activity whatever.

Chronic Lymphatic Leukemia (1 Case) and Acute Lymphatic Leukemia (1 Case): Undifferentiated oxidase-negative cells (hemocytoblasts), lymphoblasts, prolymphocytes, lymphocytes, and leukocytoid lymphocytes showed no phagocytosis. Neutrophilic cells in varying numbers showed normal phagocytic activity.

Acute Myelogenous Leukemia (1 Case), and Chronic Myelogenous Leukemia (2 Cases): Hemocytoblastic undifferentiated oxidase-positive cells show doubtful or no phagocytosis, myeloblasts very slight to doubtful phagocytosis, and promyelocytes very slight phagocytosis. A sharp increase in the phagocytic activity occurs with the myelocytes and increases to a maximum with the neutrophilic polymorphonuclears. This appears to be coincidental with the development of the mature specific neutrophilic granulations. There is practically no difference in the phagocytic ability between the mature neutrophilic polymorphonuclear and the young neutrophiles (rod-nuclears and metamyelocytes) if one takes into consideration the relative amount of cytoplasm.

Acute Hemohistioblastic (Monoblastic) Leukemia (2 Cases): The large undifferentiated hemohistioblasts (reticuloendothelial cells) show active phagocytosis, in sharp contradistinction with the undifferentiated cells occurring in acute myelogenous and acute lymphatic leukemias, which show no phagocytosis.

Monoblasts show slightly less phagocytosis. Monocytes show phagocytosis as in normal blood.

Glandular Fever (Acute Mononucleosis): The typical cells erroneously interpreted as monocytes, actually young degenerated lymphocytic cells, show no phagocytosis.

Discussion

(Dr. Jacob Furth, New York City.) May I ask whether Dr. Strumia studied normal bone marrow and compared the phagocytic activity of immature normal bone marrow cells with that of leukemic cells? Leukemic cells are probably malignant cells, and their phagocytic activity cannot be taken as an indication of what normal cells would do.

(Dr. Paul R. Cannon, Chicago.) We have made similar observations in a casual way in a patient with myelogenous leukemia. We observed the same gradient of phagocytosis for staphylococci that Dr. Strumia has found in this systematic study. We found that the immature leukocytes were but slightly phagocytic and that the degree of phagocytosis was correlated directly with the degree of maturity of the leukocytes in the leukemic blood.

(Dr. L. D. Fothergill, Boston.) I should like to mention that Dr. Diamond and I made a similar study a couple of years ago, which was not published, but which entirely confirms Dr. Strumia's results. Our study differed only in minor details. We used a rough pneumococcus, for example, instead of a staphylococcus, but our results were identical with those reported by Dr. Strumia.

(Dr. Charles Weiss, San Francisco.) May I ask Dr. Strumia whether any attempt was made to determine the relative bactericidal power of the various types of leukocytes?

(Dr. Strumia, closing.) We did quite a number of experiments with bone marrow emulsions, and also with a variety of exudative cells. The most striking one was from a sternum biopsy of a case of hemohistioblastic leukemia, the patient dying about 3 hours later. The emulsion of bone marrow was almost entirely made up of very large cells of reticuloendothelial nature, and those cells picked up everything particulate that was present in the suspension; they phagocytozed red cells and cellular fragments of all sorts, carbon granules and some collodion particles that had been lying in the laboratory for a number of years, as well as all sorts of bacteria. The phagocytic activity of these cells seemed to be less strictly dependent on the concentration of blood serum than occurs with other types of cells. I cannot make numerical statements because of the difficulty of washing these suspensions free of serum without injuring the cells. We also used an emulsion of splenic pulp from a case of acute infection showing engorgement of the sinuses with all sorts of young granulocytic cells; also emulsions of normal bone marrow, and the results seem to correspond very closely to what we presented.

In response to Dr. Weiss' question, we have made no observations whatever of the lytic power of these various cells toward ingested organisms.

OBSERVATIONS CONCERNING THE TITRATION OF VIRULENCE. Robert M. Pike (by invitation) and G. M. MacKenzie, Cooperstown, N. Y.

Abstract. The importance of conclusions to be drawn from attempts to identify with virulence some constituent of the bacterial cell seems to warrant a careful examination of the variable factors involved in the determination of virulence.

In seeking to control these factors in the titration of the virulence of *Salmonella aertrycke* for white mice, we have listed a number of variables which may affect the accuracy of the results unless properly controlled or accounted for. These variables include the different criteria in use for measuring virulence, the variables concerning the inoculum, and those concerning the test animal.

In considering the available criteria for measuring virulence, we find that accurate determination of the *minimum lethal dose* of *Salmonella aertrycke* cultures requires too many animals to be practicable, as has been shown by Lockhart and by Trevan. An analysis of the results obtained in a series of virulence tests, employing 25 mice in each test, reveals that *percentage mortality* and *mean survival time* of test animals are in general parallel although there are instances where a false idea of virulence will be obtained unless both these criteria are considered. The *mean time to death* appears, in our experiments, to be a less accurate and less reliable measure of virulence, except in tests in which the mortality is close to or actually 100 per cent.

Following the observation that the death rate increases relatively slowly with increase in the dose, it has been maintained by Wilson and by Lockhart that minor variations in the size of the inoculum, such as those that result from inaccuracies in technic, have no significant effect on the result. With most cultures, especially those of intermediate and low virulence, we find, however, that there is a critical range of dosage in which doubling the dose may decrease the survival time as much as 30 per cent. Accurate standardization of the inoculum is therefore of utmost importance.

The danger of drawing conclusions in regard to virulence from the results obtained with very small numbers of animals is again emphasized.

PHAGOCYTOSIS OF HEMOLYTIC STREPTOCOCCI. Stuart Mudd and (by invitation) Horace Pettit, David Lackman and E. J. Czarnetzky, Philadelphia, Pa.

Abstract. Phagocytosis of hemolytic streptococci by washed exudative rabbit leukocytes in the presence of serial dilutions of serums has afforded a sensitive analytical method. The phagocytosis-promoting effects of serums in general have paralleled their mouse protective values. Phagocytosis and protective effects are type-specific for Griffith types, but with some minor cross reaction.

The "C" substance as prepared by Lancefield's method has been found to contain a certain proportion of another antigen which gives a precipitate with any antiserum prepared against the *Beta*-hemolytic streptococci. This "C" substance which has been further purified (C′ substance) has given precipitates with serums only against certain types of Group A. Absorptions of Griffith Type I antiserums with the fractions of Lancefield's "C" substance, and with "M" substance from a homologous strain fail to reduce their phagocytosis-promoting value; indeed such absorptions increase phagocytosis, possibly because of removal of inhibiting serum lipoids.

A heavy suspension of hemolytic streptococci may be thoroughly disintegrated by exposure for 1 hour to intense sonic vibration produced by a magnetostriction oscillator of design described by Chambers and Gaines (*J. Cell. & Comp. Physiol.*, 1932, 1, 451), and Chambers and Flosdorf (*Proc. Soc. Exper. Biol. & Med.*, in press). When the bacterial débris has been thrown down by centrifugalization, the decanted supernatant fluid is found to contain a solute which will completely absorb the phagocytosis-promoting antibody from homologous rabbit antiserum.[1]

Acidification with hydrochloric acid to certain characteristic pH values causes precipitation of a fraction (designated P) from the supernatant fluid from sonic disintegration of streptococci. This fraction has the property of precipitating the phagocytosis-promoting antibody from the specific antiserum. The neutralized fluid remaining after such acid precipitation is inactive against the phagocytosis-promoting antibody. The supernatant fluid from the sonic disintegration may be passed through a Berkefeld filter and preserved by the lyophile process without losing its activity.

This newly discovered component is exceedingly labile. It has been deprived of most or all of its antiphagocytic activity by: (*a*) heating to 56° C. for 30 minutes; (*b*) keeping in 1 per cent formaldehyde at neutral reaction for 10 minutes; and (*c*) keeping at pH 10.0 for 10 minutes.

An acid reaction, pH 2.0, for 10 minutes only slightly reduced the activity of the labile substance. Experiments are under way to test the capacity of this labile bacterial component to elicit active immunity in mice and rabbits.

CHEMOTROPISM OF LEUKOCYTES: THE SOURCE OF SUBSTANCES ATTRACTING MONOCYTES TO BACTERIA. Harold M. Dixon (by invitation) and Morton McCutcheon, Philadelphia, Pa.

Abstract. Earlier observations showed that human polymorphonuclear leukocytes, as observed with the microscope *in vitro*, were strongly attracted by all of a number of diverse types of microörganisms, with nearly equal intensity, as computed from the direction of the leukocyte's paths. The nearly uniform reaction of leukocytes to different microörganisms suggested a chemotropic factor common to all. This factor was apparently not the size of the bacteria, since carbon particles of approximately the same size as bacteria excited no reaction on the part of leukocytes. The culture medium was next investigated, since all the microörganisms were taken from artificial media, from which substances attractive for leukocytes might adhere to the bacteria. It seemed possible that such substances, if present, could be removed from the bacteria by repeatedly washing them with NaCl solution or distilled water, but this procedure failed to decrease their attraction for leukocytes. Further experiments were then made to decide whether the culture medium or the bacteria themselves were the source of the attraction. A drop of infusion broth dried on a glass slide failed to attract leukocytes. *B. coli* were then grown in broth, which was subsequently passed through a Berkefeld filter. A drop of the bacteria-free filtrate was found to attract leukocytes with moderate intensity. It was therefore concluded that it was the bacteria rather than the culture medium that attracted leukocytes. Experiments were then designed to answer the question whether, *in vitro*, bacteria directly attract leukocytes, or whether they do so indirectly through injuring leukocytes which themselves then give off attractive substances. It was found that when leukocytes were trapped on the margin of a mass of bacteria and became immobile, other leukocytes did not pile up on the trapped cells but arranged themselves at random on the edge of the bacterial clump, as would be expected if the bacteria attracted them directly. From these and other experiments it is concluded that under the present conditions chemotropism to bacteria is due to some substance or substances which are given off by the bacteria and which excite a directional response on the part of the leukocyte.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I am highly interested in the experiments related here, because they have a direct bearing on certain phenomena associated with inflammation, particularly that due to bacterial or infectious agents. In experiments on inflammation not incited by bacteria, but resulting from a sterile burn of the skin, we noticed that leukocytes were drawn in large numbers and very early to the area of injury. In this experiment no bacteria of any kind were concerned. The results indicated that some substance derived from the injured cells attracted bacteria to the area of injury. I should like to know whether Drs. Dixon and McCutcheon have tried the chemotropic effect of injured tissue, as epidermal cells or other injured cells, for leukocytes.

(Dr. Dixon.) In answer to Dr. Moon's question, leukocytes were taken from the buffy coat of human blood, dried, and then tested for chemotropic substances. We found that other leukocytes were attracted very weakly by the dried cells.

(Dr. Moon.) This would indicate that the leukocytes themselves do not give forth a chemotropic substance, but it does not indicate that the other cells of the body when influenced by injury, may not give forth a chemotropic substance. It seems to me that similar experiments should be tried on tissue cells.

SERUM ESTERASE IN RATS INJECTED INTRAPERITONEALLY WITH FRESH MACERATED TISSUES.* F. A. McJunkin and (by invitation) G. A. Hemwall and E. A. Fullgrabe, Chicago, Ill.

Abstract. Although Shaw-Mackenzie (*Proc. Physiol. Soc., London*, 1911, **42**, 11) in 1911 observed in cancer-resistant mice an increased capacity of the serum to activate lipase, Green (*Brit. J. Exper. Path.*, 1934, **15**, 1) appears to have been the first to determine the ester-hydrolysing property of serum in mammalian malignancy. However, Falk (The Chemistry of Enzyme Actions, 1934, Ed. 2, N. Y.) had studied the esterase content of a number of normal and malignant tissues and states: "The first striking fact which may be mentioned is the small value of the carcinoma (Flexner-Jobling) on all esters." Green found a definite fall in the esterase content of the serum even at a very early stage of the tumor (Jensen sarcoma) growth. Sure, Kik and Buchanan (*Biochem. J.*, 1935, **29**, 1508) working with Walker rat tumor No. 256 have confirmed the findings of Green. Directly following Green's publication 2 years ago we made esterase determinations on a number of rats inoculated with a spindle cell sarcoma which was primary in the uterus and which yields 90 per cent of positive "takes" when inoculated by the pocket method. The Green

* Since the above report was made additional results have been obtained. Cholesterol has been found to reduce esterase to a much greater extent than any of the other constitutents of the tumor which were tested. The esterase in 22 rats which received an average dose of 133 mg. of cholesterol was reduced to 13.5, a decrease of 9.6 or 37.9 per cent after allowing for the "bleeding effect." Since such small doses of cholesterol, entirely without toxic effects so far as could be determined, reduce the esterase even more than 2 gm. of fresh tumor tissue, it seems to us that the cholesterol content of tissues may play a prominent rôle in the reduction of esterase that follows macerated tissue injections, and that the reduced esterase activity of sarcoma-bearing rats may be explained by absorption of cholesterol from the tumors.

technic was followed with the notable exception that the samples of blood were withdrawn from the heart of the living rat without the use of anesthetic. In 211 normal rats (weight 150 to 275 gm.) the serum had an average esterase content of 25.3 (cc. N/100 NaOH to neutralize fatty acid liberated from ethyl butyrate). In 20 sarcoma-bearing rats (average age of tumor 28 days) the esterase was 13.5, a decrease of 11.8 (46.6 per cent) below the normal mean. We then sought an explanation for the esterase reduction by injecting constituents of the tumor and of rat tissues. Fats (olive oil), fatty acids (oleic acid), and phospholipids (lecithin (Eastman practical)) were injected. Serum esterase determinations were made 24 hours after the injections. Of these only lecithin in large doses (650 mg.) brought about an esterase reduction which amounted to only 3 after allowing 2.2 for the "bleeding effect." To determine the "bleeding effect" blood (2–3 cc.) was withdrawn from the heart of 52 normal rats and 24 hours later samples were again removed. The esterase average of the second samples, which represents the effect of a single bleeding, was 2.2 less than that of the initial ones. Attention was turned to the non-lipoid portions of the tissues and fresh macerated sarcoma was injected. Two gm. of the fresh macerated tumor injected intraperitoneally caused an esterase decrease of 32.4 per cent after allowing for "bleeding effect." Four gm. of fresh macerated rat liver resulted in a decrease of 24.9 per cent after allowing for "bleeding effect." In a small group of 8 rats large doses of Witte's peptone which approached the lethal dosage reduced the esterase 35.7 per cent after allowing for "bleeding effect." Smaller doses produced no regular effect. Injections of fresh macerated rat sarcoma result in esterase reductions comparable to the decreases observed in rats bearing large tumor growths. It is quite possible that absorption of protein split products from the autolyzing tissues and of phospholipids may account for the esterase decreases. However, it is felt that additional experiments are required to explain the results.

MYASTHENIA GRAVIS: AUTOPSY FINDINGS IN A CASE. Charles F. Branch, Boston, Mass.

Abstract. A case of myasthenia gravis in a white adult female 23 years of age is presented. The clinical course was characteristic. The autopsy findings were essentially similar to those reported by other observers with the following exceptions: No thymic tumor was present; on the contrary, a distinct atrophy of the thymus was noted. No central nervous system lesions were found. Numerous minute, acute inflammatory lesions were found in the striated and cardiac muscle, associated with the usual lymphocytic infiltration described by others. The author was unable to demonstrate the presence of streptococci in these lesions.

Discussion

(Dr. Edward C. Rosenow, Rochester, Minn.) In Dr. Butt's work, when he stained his sections by the usual Gram method, he found no organisms. When he stained them in like manner, but only partially decolorized them, a method which we have used successfully many times in other studies, he obtained diplococci in all of his cases. We have studied 49 patients having myasthenia gravis and we have found consistently a streptococcus in the nasopharynx, tonsils, infected teeth, urine, and excised muscle which, when injected into monkeys and rabbits, localized selectively in muscles. If large doses were given, there was a

primary reaction in which weakness was a pronounced symptom, following which apparent recovery ensued, which in turn was followed long after injection by progressive weakness and fatigability, and finally great loss of weight. Cultures taken from the muscles after death have yielded streptococci with great regularity, whereas cultures from the blood and other viscera were nearly always sterile. Sections of the muscles of monkeys and rabbits which developed symptoms resembling myasthenia gravis revealed lymphocytic collections and other lesions resembling those of myasthenia gravis.

(Dr. Branch, closing.) In staining our slides we used the method suggested by Dr. Butt.

BLASTOMYCOSIS OF THE HEART. R. D. Baker and E. W. Brian (by invitation), Durham, N. C.

Abstract. Blastomycosis of the heart was encountered at autopsy in 2 cases of generalized infection with *Blastomyces dermatitidis*. Each showed diffuse pericardial blastomycosis, a large blastomycotic tubercle of the right atrial wall, and involvement of the corresponding endocardium. From the latter site organisms apparently entered the blood stream to produce the miliary pulmonary blastomycosis noted in both cases. Evidences of cardiac insufficiency, dependent probably on the cardiac blastomycosis, occurred in both.

Blastomycosis of the heart may also develop as part of a generalized miliary blastomycosis, and possibly by retrograde lymphatic extension from the infection in mediastinal nodes.

Blastomycosis is similar to tuberculosis in respect to cardiac involvement.

EMBOLIC PULMONARY LESIONS PRODUCED IN RABBITS BY HUMAN FAT CONTAINING FATTY ACIDS OR SOAPS (CA. SR. BA.). Edwin F. Hirsch, Chicago, Ill.

Abstract. The fatty acids of oil systems in contact with alkaline aqueous solutions exchange H-ions for the basic ions dissolved in the aqueous fluid.

Fatty acids in such oil systems are solvents for certain soaps insoluble in aqueous solutions.

Human fat containing fatty acids abstracts base ions from contacting aqueous liquids and the soaps so formed are important in determining the subsequent tissue reactions.

The exchange of H-ions and base-ions at the oil-water interphase when an oil contains dissolved fatty acid is an important link between the chemical reactions of the two immiscible systems.

Human fat with a comparatively high content of oleic or stearic acid produced in the lungs of rabbits a marked fibroblastic tissue reaction, the stearic acid mixture some large foreign body giant cells.

Human fat containing smaller concentrations of oleic and stearic acids neutralized with calcium hydroxide stimulated leukocytic exudates and a moderate fibroblastic tissue response. After neutralization with strontium or barium hydroxide it stimulated a marked fibroblastic tissue, epithelioid and giant cell reaction.

The complete report will be published in the *Archives of Pathology*.

POSTOPERATIVE PULMONARY EMBOLISM. J. S. McCartney, Minneapolis, Minn.

Abstract. In a series of 12,500 consecutive autopsies, death in 2058 instances was classified as postoperative. Of the deaths so classified, 1303 were in males and 756 in females, a ratio of 1.7 to 1. The number of fatal pulmonary embolisms in males was 64 and in females 41 or percentage incidences of 4.9 and 5.4 respectively. When adults only were considered, these percentages became 5.6 and 5.7.

Examination of the records according to the site of operation showed no significant difference between abdominal and extra-abdominal operations as forerunners of pulmonary embolism. Eighty-three embolisms followed abdominal operations and 22 followed extra-abdominal operative procedures. In the group of abdominal operations there was a marked difference in the number of embolisms following surgical procedures on the various organs. Operations on the prostate and bladder led with 18 emboli, herniotomies accounted for 14 and operations on the uterus and adnexae for 12. Herniotomy showed the highest percentage, 19.4; the prostate and bladder next, with 11.7; and the incidence was 10.3 following abdominal operations on the uterus and adnexae. It was of interest that the incidence following herniotomy was 24 in males and 9.1 in females. The incidence following appendectomy was 6 per cent. Sixty-six operations on the perineum and external genitals caused 7 fatal embolisms.

Comparison of the age distribution of the cases showed almost identical percentages for the two sexes in the different decades. However, a similar comparison of the age distributions of the embolisms showed apparent sex differences, the embolisms appearing at an earlier age in females but lasting to a later age in males. A possible explanation for this was the fact that operations on the uterus and adnexa took place for the most part much earlier in life than did operations on the prostate and bladder. Herniotomies occupied a position between these two sites.

Discussion

(Dr. Edward C. Rosenow, Rochester, Minn.) I should like to ask whether there was any seasonal incidence in the number of postoperative deaths from pulmonary embolism.

(Dr. McCartney, closing.) There was no evidence of any seasonal distribution at all.

PNEUMONIA IN NEWBORN INFANTS. Margaret Warwick, Buffalo, N. Y.

Abstract. In 420 consecutive autopsies on babies who were stillborn or died within the first 10 days of life, 32 were too macerated for detailed examination, but of the remainder, 77 or 18.7 per cent showed pneumonia. Of the 77, 27 or 48 per cent lived less than 24 hours, and 54 or 70 per cent lived less than 48 hours, showing that the condition was closely related to birth. All of the lungs were stained for bacteria which were present in 22 or 30 per cent. This number increased with age and, among those living less than 24 hours (37), only 3, or 8 per cent, showed bacteria, suggesting that they might be introduced after birth by artificial respiration or by aspiration. The possibility of bacteria having been introduced after premature rupture of the membranes was excluded by the fact that 49, or 63 per cent, had had the membranes ruptured less than 10 hours before birth, and 56, or 73 per cent, had ruptured less than 24 hours before.

The majority of these infants had had difficult births because only 17, or 22

per cent, had been born by a spontaneous delivery and of these, 5 had had unusually long labors. Only 11, or 14 per cent, were recorded as "good" at birth and 49, or 63 per cent, were dead or "poor" and the rest were "fair." Of the 77, only 16 died without other pathological lesions, such as malformations or birth injuries, sufficient to have caused death without the pneumonia. Therefore this pneumonia seems to be associated with the process of birth.

Infants frequently have premature respirations before or during birth due to asphyxia from some disturbance of the circulation of either mother or child, and this sucks amniotic fluid containing cornified epithelial cells and often bile pigments from meconium deep into the lungs. Of these 77 pneumonia cases 65, or 84 per cent, showed the cornified epithelial cells and bile salts to be present, while in the remaining 12, or 16 per cent, the pus cells were numerous enough to have obscured the evidence. So it seems that these irritating substances, which are foreign bodies, may set up an inflammatory process identical with a "chemical" pneumonia which would not have a toxic effect but would mechanically fill the alveoli and prevent proper aeration. An infant would probably have premature respirations during a long and difficult delivery and, if it were born in a poor condition, would lack the energy and vitality to expel the amniotic fluid so that much would remain to cause a pneumonia. A few may have the pneumonia already established at birth, if they have breathed long before.

Therefore it seems that the pneumonia which is found in about one-fifth of all infants dying during the first 10 days of life is the effect of a hazardous birth and not an infectious process and is more commonly found in weak infants, or those who have been maltreated during birth.

Discussion

(Dr. Herbert S. Reichle, Cleveland.) Has Dr. Warwick any statistics as to whether there were any areas preferred by the process in those cases where the disease was not diffuse throughout the lungs?

(Dr. Max M. Strumia, Philadelphia.) I should like to ask what criterion was used to determine the presence of bacteria in the lungs? Did you use stained smears only?

(Dr. A. W. Wright, Albany.) I should like to ask a question very much like that of the previous speaker, namely, did Dr. Warwick carry out blood culture studies in the case of any of the infants, and if so, did such cultures show anything of significance? I also have a second question. If the pneumonic process was recognized at the time of gross examination of the lungs, were cultures from these organs taken at that time? Dr. Warwick's percentage of acute pneumonias in young infants is considerably higher than ours. We have on certain occasions found that the blood has contained pathogenic bacteria while there was no evidence at all of a pneumonic process. We have also seen, as Dr. Farber and his colleagues have, a considerable number of lungs that contained aspirated amniotic fluid and desquamated squamous epithelial cells without any evidence of a marked inflammatory reaction such as we saw in some of the photomicrographs shown by Dr. Warwick.

(Dr. Irving Graef, New York City.) I should like to ask if Dr. Warwick has taken into consideration the possibility that the polymorphonuclear leukocytes found in the lungs were also aspirated from the amniotic sac, reflecting inflammation of that structure. In the material at Bellevue Hospital from the obstetric and pediatric services, it is also common to find cases in which the

aspiration of amniotic fluid, indicated by the presence of epidermal cells and meconium, is not associated with polymorphonuclear leukocytes. Therefore I feel sure we are not justified in concluding that these normal elements of the amniotic fluid necessarily incite an inflammatory process.

(Dr. Warwick, closing.) In reply to Dr. Reichle, there seemed to be no preferred areas except around the bronchi, where we nearly always found the areas of pneumonia. We found many of them in the upper lobes and many in the lower, while a great many were diffuse throughout the lung.

Our only criterion for the presence of bacteria was staining, and we carried that through with controls. No blood cultures were taken, and no cultures were made of the lungs themselves. Unfortunately in our hospital it is difficult to get autopsies at once on these babies. We try to get permission for them, which is at first refused, and then all the way from 6, 8, 12 or even 24 hours later the families will consent to the autopsy, so we do not have proper culture material. But I have cultured several cases without finding anything.

These pneumonias were not recognized clinically in the majority of cases, because the pediatrician called them atelectasis. A few of them had X-ray pictures, and they noted what they called atelectasis, which gives the same X-ray picture as this pneumonia.

In regard to Dr. Graef's suggestion as to the possibility of the leukocytes coming from the amniotic fluid, we found a good many of these young infants in the series that showed aspirated amniotic fluid without showing pneumonia, that is, the criterion of the epithelial cells and the bile salts. These were very distinct; they were usually pretty large in size; they did not look like bacteria; they had very different shapes and a different color, and with the ordinary oil immersion lens it seems to me that the bile salts could not be mistaken for bacteria, or the other way around. It is certainly evident that we have large numbers of infants who aspirate amniotic fluid who do not show polymorphonuclears in the lungs, in the alveoli or bronchi, and it seems to me there is the possibility that had some of these infants had a little more strength to get rid of the amniotic fluid shown by the small areas of lung infected, and had they not had other hazards to their early life, they might very easily have not succumbed to this pneumonia. I do not think it is toxic; I do not say it is the cause of death, except from the mechanical point of view by filling up the lungs and preventing proper aeration. I will admit the bacteriological studies are not sufficient, but our stains for bacteria are satisfactory, and it seems very easy to recognize them, and as I said before, they were stained with controls from pneumonia in adults.

NEOPLASTIC CHANGES PRODUCED IN THE OVARY AND OTHER ORGANS OF MICE BY IRRADIATION OF THE ENTIRE BODY WITH X-RAYS. J. Furth and (by invitation) J. S. Butterworth, New York City.

Abstract. The incidence of most spontaneous neoplasms in mice is greatly increased by massive irradiation at the age of from 2 to 3 months; the incidence of breast cancer is decreased.

Lymphomatosis		Myelosis	Tumors				Negative
Mediastinal	All other types		Breast	Ovary	Lung	Miscellaneous	
Irradiated mice (374 ♀, 405 ♂):							
9.1%	8.0%	7.1%	5.9%*	17.4%*	10.7%	3.7%	55.2%
Control mice (708 ♀, 567 ♂):							
1.3%	4.0%	0.9%	11.6%*	1.1%*	7.1%	1.7%	78.3%

* Of females: all other percentages are those of all mice.

The ovarian tumors are granulosa-celled, adenoma-like and lutein-celled growths. Some of them are associated with cystic hyperplasia of the endometrium (in 1 case, endometriosis), and hyperplasia of the anterior lobe cells of the pituitary (in 1 case adenoma-like).

Discussion

(Dr. Kornel Terplan, Buffalo.) I should like to ask two questions about this very interesting demonstration: were there any hematological blood studies made on the mice previous to the appearance of the tumors? The reason I ask this question is because we may see in man following severe damage to the bone marrow a severe anemia, and almost complete disappearance of the granulated elements, which may later be followed by acute myelogenous or lymphatic leukemia. In one of these cases which I saw there was a combination of lymphatic leukemia with blastomatous proliferations of reticulum cells.

My second question is whether Dr. Furth or any other member of this Society ever happened to see radiation, especially roentgen-ray treatment of epithelial tumors, followed by the appearance of a sarcoma at or near the site of the apparently healed carcinoma. I remember a case with a primary sarcoma following many years of extensive X-ray treatment of a basal cell carcinoma of the face. This sarcoma was very close to a diffuse hyalinized scar which was the remainder of the X-ray-treated basal cell carcinoma.

(Dr. David P. Seecof, Montreal.) Are these animals which are irradiated and which develop tumors of the ovaries sterile, or do they ever have any litters? Is the X-ray dose sufficient to sterilize them for the remainder of their lives?

(Dr. Furth, closing.) In regard to Dr. Terplan's question, the dose used produces profound atrophy of the bone marrow with leukopenia. Leukemia and in general the tumors mentioned arise in organs that were severely injured by the X-rays. The appearance of neoplasms follows regenerative processes.

It is conceivable that radiation of a tumor produces another neoplasm, but one would expect the second neoplasm to appear after a very long incubation period. Long latency is characteristic of experimental cancer.

To answer Dr. Seecof's question, the ovary remains free from ova for the rest

of the animals' lives. This is significant in relation to the discussion whether there is postnatal ovum formation or not. We found excessive regeneration of all other elements of the ovary, but have never seen an ovum in irradiated ovaries.

THE CELL FACTORS INFLUENCING VIRUS-INDUCED PAPILLOMAS OF THE RABBIT. John G. Kidd (by invitation), New York City.

Abstract. The rabbit papillomas caused by the Shope virus are formed by the multiplication of the epidermal cells originally infected with the virus. These growths vary in their course, some enlarging progressively, while others retrogress. Both in their enlargement and retrogression the growths offer opportunities for the study of the part played by the cells in a prolonged virus-cell association that gives rise to growths of neoplastic character.

The activity of the virus material is of prime importance in determining the course of the growths, but host factors exercise an influence which is frequently decisive. Multiple papillomas produced with the virus in any one individual run the same general course when this is not complicated by the influence of intercurrent local factors, but from host to host the variation in size and fleshiness is considerable. The growths are generally larger and fleshier in rabbits whose skin proliferates actively in response to the injection of scharlach R, dibenzanthracene, tar extracts, and certain other stimuli. When virus suspensions from several different wild rabbits are tattooed into many spots on the skin of each of a group of comparable domestic rabbits, the growths that result from the different inoculums may differ considerably in time of incubation and rate of enlargement, yet those of a single host all tend to wax or wane together. In some animals they enlarge progressively, whereas in others they grow well for a time and then retrogress. Evidently some general influence of host derivation determines their fate. This influence is distinct, however, from that of the principle neutralizing the virus which appears in the blood of rabbits bearing the papillomas, for this principle does not influence the course of the growths significantly. Experiments have shown that the papillomas grow steadily in animals whose serums have marked capacity to neutralize the virus *in vitro*, and good evidence exists that the cells protect the virus stimulating them.

Many transplanted tumors call forth a resistance on the part of the host which is directed against the proliferating cells. Can it be supposed that a similar phenomenon is elicited by the papilloma during its growth, the resistance in this case being directed against the animal's own virus-infected cells? Certain facts speak in favor of this conception. In three groups of comparable domestic rabbits, 4, 24, and 64 discrete papillomas respectively were produced by tattooing virus into the skin of the sides. The incidence of retrogression was the same in all of the groups, but the larger the number of papillomas the sooner and more rapidly did it occur. Evidently the host's own proliferating, virus-infected cells, or some product of these, acted to elicit a host resistance, the effectiveness of which varied with the amount of papilloma tissue calling it forth. The resistance, however, was not always completely effective. In several exceptional instances small growths retrogressed completely, whereas larger ones of the same host, though dwindling when the others did, did not wholly disappear but ultimately enlarged again and later grew progressively. Whatever the nature of the defense mechanism responsible for retrogression, it is evidently subject to fluctuations in effectiveness.

In its histological features the retrogression of the papillomas resembled that of epidermal tumors generally, notably the tar papillomas — a fact which supports the view that it may be due to similar influences exerted upon the cells. The growth becomes more orderly, hair follicles and sebaceous glands reappear beneath it, its processes become narrower, and almost insensibly their place is taken by normal epidermis. Confluent papillomatous masses take much longer to retrogress completely than small discrete growths, the process going on principally at their periphery. Local stimulation of the papilloma cells by bacterial infection or scharlach R not only makes vigorous papillomas grow faster but may check and, for a time at least, prevent retrogression. The reactive inflammation present in the large masses acts to favor their progressive growth.

It seems likely that the host resistance which not infrequently overcomes rabbit papillomas is elicited by and directed against the animal's own proliferating virus-infected cells. The frequency of retrogression can be referred to the unfavorable character of many of the rabbits in which papillomas are experimentally induced.

NESIDIOBLASTOMA, THE ISLET CELL TUMOR OF THE PANCREAS. George F. Laidlaw and (by invitation) Virginia Kneeland Frantz, New York City.

Abstract. A study of nine adenomas of the islets of Langerhans removed surgically from six patients by Dr. Allen O. Whipple, at the Presbyterian Hospital, New York City, is presented. In each instance there followed prompt disappearance of the hypoglycemia with its collateral symptoms, for the relief of which the operations had been performed. The tumors were small, varying from 4 mm. to 2 cm. in diameter.

Microscopically the chief feature of most of the tumors is their exact duplication of the pattern of normal islets, consisting of longer or shorter ribbons of cells bordering on a rich plexus of capillaries. Cellular details are revealed best by fixing in Zenker's fluid, staining paraffin sections with acid fuchsin and differentiating in methyl green. In normal pancreas, so treated, the acinus cells are green with green nuclei, zymogen granules red, basal filaments and other mitochondria red. In contrast with the green acinus cells, the islet cells are packed with bright red granules. With a slight modification of the technique, the granules of the *A* cells hold the red; the granules of the *B* cells turn purple. The tumor cells react to this stain exactly like islet cells. In most of the tumor cells the granules take the purple color of *B* cells with here and there a red *A* cell. In the tumors, we have not found Bensley's granule-free *C* cells or the *D* cells which stain with aniline blue.

The tumors resemble islet hypertrophies in their tendency to exaggerate some feature of the normal islet pattern. For instance, Tumor 2 repeats over and over and exaggerates the rosette formation around the blood channels which is an occasional figure in normal islets. Tumors 5 and 6 consist almost entirely of long ribbons of large columnar cells with centrally placed nuclei, exaggerating and repeating a feature sometimes seen in normal islets, but more frequently in their hypertrophies. Tumor 1 copies the usual compact endocrine pattern so exactly that even with the highest magnifications one cannot distinguish it from a normal islet except at the margin where it compresses the surrounding pancreas, and from its size. It is really one gigantic islet 1 cm. across.

Just as the tumors duplicate the structure of normal and hypertrophied islets, so are they subject to the same pathological vicissitudes, especially fibrosis,

hyaline degeneration and calcification. We agree with other observers that the fibrosis begins along the capillaries as a fibrous thickening of the capillary wall with projection of small blocks of collagen encroaching on the tumor cells. Six of our tumors show extensive fibrosis. Much of the newly formed fibrous connective tissue has been converted into a clear glassy substance which, in the negative outcome of amyloid and mucin reactions, we must be content to call hyalin.

With Mallory's aniline blue connective tissue stain or with its variants, Masson's trichrome and Heidenhain's azocarmine, the hyaline substance stains pale blue, much paler than the fibrous connective tissue. Many tumor cells show similar pale blue patches in their cytoplasm. Studying these patches, we are convinced that the tumor cells themselves undergo the hyaline change as well as the fibrous connective tissue, settling in our own minds at least the long-standing controversy as to whether the hyaline metamorphosis is restricted to the collagen or to the cytoplasm. It affects both.

In some of our tumors the origin of the tumor cells is indicated by figures where the epithelial lining of a duct is continuous with a group of tumor cells. Here again we observe an exaggeration of a normal procedure. In the normal pancreas, continuity of duct cells and islet cells is a common observation. It is admitted by all that the duct cells are totipotent, producing acinus cells and islet cells during embryonic life and probably throughout adult life as well. If the cells of the islet tumors differentiate out of duct epithelium, they are merely exaggerating the normal procedure of islet building, probably with coincident formation of many new ducts.

The name *nesidioblastoma*. There is need for a short and accurate name for these tumors. Adenoma of the islets of Langerhans is long and cumbersome. Adenoma itself is vague, for we have already two kinds of adenoma, the benign epithelial tumor and lymphadenoma, quite different from each other. Another adenoma, an endocrine kind, merely adds to the confusion. We have followed current custom of suffixing "oma" to the Greek name of the cells of origin of the tumor. Selecting *νησίδιον* as the Greek word for islet, the cells that differentiate out of the duct epithelium to build islets may be called nesidioblasts, or islet builders. When these islet builders or nesidioblasts form tumors, the tumor is a nesidioblastoma. The name has another application. In contrast with the concentration of excess islet tissue in a tumor, evidence is accumulating of a diffuse or disseminated proliferation of islet cells as a possible cause of hypoglycemia. Such a proliferation of nesidioblasts would be a nesidioblastosis.

LIPOSARCOMA OF BONE: A REPORT OF TWO CASES. Donald J. Rehbock and Harry Hauser (by invitation), Cleveland, Ohio.

Abstract. The 1st case presented is that of a woman 56 years of age who had a pathological fracture of the right femur, followed in 7 months by a pathological fracture of the left femur. The patient died 15 months after the onset, and was found at autopsy to have generalized tumor metastases, the primary site being in the right femur. The histological diagnosis was liposarcoma.

The 2nd case was that of a 60 year old white man who was seen only shortly before death, and who had had symptoms for 1 year. At autopsy there was found a large tumor involving the right ilium and adjacent sacrum. There were no tumor metastases. The histological diagnosis was liposarcoma.

These cases are No. 1904 and No. 1224, respectively, of the American College

of Surgeons Bone Sarcoma Registry. A review of the literature reveals only 5 other recorded cases of primary liposarcoma of bone.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Rehbock how he distinguishes this neoplasm from the Ewing tumor of bone. It has the same general features as a Ewing tumor. The fat in the cells might be merely a degenerative process which we often see in malignant tumors. There is no direct evidence of a differentiation into adipose tissue cells.

(Dr. S. Burt Wolbach, Boston.) I was about to suggest that tumors be stained with fat stains more frequently, because I think we would find fat much more often than expected. That has been our experience with some tumors that obviously could not be regarded as lipomas — including epithelial tumors.

(Dr. Kornel Terplan, Buffalo.) I should like to ask whether one of the tumors shown could not be called a reticulum cell sarcoma. This type is not uncommon as a primary blastoma of the bone marrow. The small fat droplets which were seen in giant cells may have been phagocytosed from the fatty marrow in the bone.

(Dr. Rehbock, closing.) The diagnosis of liposarcoma here does not rest entirely on the presence of fat within the cytoplasm of the cells. The cells must show no evidence of nuclear or cytoplasmic degeneration. It is true we did find fat in areas which obviously show degeneration, but we did find fat in the undegenerated cells. The cellular morphology is much different from that of the Ewing tumor and reticulum cell sarcoma. The morphology of the cells, I believe, is a more important clue to the diagnosis than the presence of fat, — large cells with abundant cytoplasm, resembling the embryonic type of fat cell.

BONE MARROW PICTURES IN THE ANEMIAS AS STUDIED BY STERNAL BIOPSY. R. P. Custer, Philadelphia, Pa.

Abstract. Biopsy of the sternal bone marrow has been practiced with increasing frequency at the Philadelphia General Hospital during the past 4 years and has served (1) to give the patient the benefit of more accurate diagnosis and prognosis; (2) to afford the attending clinician firmer ground on which to base his therapeutic measures; and (3) to catalogue more adequately the various disorders of the blood-forming organs and accumulate further data for the study of these disturbances.

A 1 cm. diameter button of bone is removed from the ventral table of the sternum by trephine under local anesthesia; imprints and streaks are stained by Pappenheim's May-Grünwald-Giemsa method. The button is fixed in Helly's fluid, decalcified in formic acid-sodium citrate solution, sectioned in paraffin at 4 microns and stained by a modification of Maximow's azure II eosin method. Differential counts of 500 cells are made by the method previously described (Krumbhaar and Custer).

A small chapter from the accumulated data is presented, depicting differences and similarities of the bone marrow picture in a few selected types of anemia as shown by matched photomicrographs and differential counts. Abbreviated counts from the group follow:

		Hemorrhagic anemia	Pernicious anemia			Normal	Idiopathic aplastic anemia	Idiopathic hypochromic anemia	Parasitic anemia (uncinariasis)	Sickle cell anemia
			Relapse	Early remission	Late remission					
UNDIFFERENTIATED CELLS		1.8	2.4	0.6	0.4	0.0	0.8	2.6	1.2	2.4
MYELOBLASTS		0.2	0.8	1.0	1.4	0.6	0.0	0.8	0.4	0.8
PROMYELOCYTES	neutrophil	2.4	0.8	1.6	6.6	9.0	0.4	1.8	1.8	1.8
	eosinophil	0.0	0.0	0.4	0.6	0.0	0.0	0.0	1.2	0.0
MYELOCYTES	neutrophil	5.4	3.4	4.0	15.4	34.6	0.8	7.4	6.0	8.2
	eosinophil	1.6	2.8	3.0	2.0	2.0	0.2	0.8	5.2	0.8
METAMYELOCYTES	neutrophil	8.4	4.6	5.8	9.2	14.6	1.1	9.8	9.0	10.8
	eosinophil	0.8	1.2	2.6	1.0	0.0	0.2	1.2	2.6	0.4
SEGMENTERS	neutrophil	3.4	10.2	8.4	6.8	2.9	0.0	5.4	5.4	4.8
	eosinophil	0.2	3.2	1.8	0.8	0.1	0.0	0.0	1.6	0.2
TOTAL		22.4	27.0	28.6	43.8	63.8	2.7	27.2	33.2	27.8
PROMEGALOBLASTS		1.8	2.8	1.0	0.8	0.0	0.2	4.4	4.6	1.2
MEGALOBLASTS		0.6	38.6	8.2	3.6	0.0	0.4	3.8	3.2	2.4
ERYTHROBLASTS		33.6	13.8	47.2	22.2	14.8	45.2	34.4	31.8	33.4
NORMOBLASTS		32.0	5.4	10.1	27.2	18.2	30.4	20.2	23.2	24.0
TOTAL		68.0	60.6	66.5	53.8	33.0	76.2	62.8	62.8	61.0
MEGAKARYOCYTES		1.2	0.8	0.8	1.0	1.0	0.2	0.4	0.4	0.8
RETICULAR CELLS		3.2	5.2	1.6	0.8	1.2	11.0	5.0	1.4	4.0
ENDOTHELIAL CELLS		3.4	4.0	1.6	0.2	1.0	9.2	2.0	1.0	4.0

Biopsy should be performed whenever possible before treatment of any kind has been instituted, especially liver administration. This is well shown by the example of early remission in pernicious anemia in which the patient was given a single dose of liver extract parenterally 2 days before biopsy; striking changes in the marrow obviously precede changes in the peripheral blood.

While a scattering of promegaloblasts and megaloblasts appears in the marrow in practically any type of severe anemia, erythropoiesis is essentially of the so-called normoblastic type in all varieties shown here except pernicious anemia in relapse; in aplastic anemia a great gap lies between stem cells and late nucleated red cells, the few megaloblasts being confined to tiny foci of attempted regeneration. In sickle cell anemia erythropoiesis is normal save for a very occasional sickled normoblast.

While the bone marrow picture may occasionally be inconclusive, it is much less often so than studies on the peripheral blood alone, and the practice of sternal biopsy deserves wider use than it is now accorded. Emphasis is again laid on the value of carefully prepared sections in conjunction with smears, as opposed to study of smears obtained by simple sternal puncture.

Discussion

(Dr. S. Burt Wolbach, Boston.) I must say I congratulate Dr. Custer on the size of the trephine that is used in Philadelphia. I wish we could persuade them to use similar trephines in Boston hospitals.

THE PRODUCTION OF PERSISTENT HYPERTENSION IN MONKEYS. Harry Goldblatt, Cleveland, Ohio.

Abstract. By the constriction of the renal arteries by means of a special silver clamp, smaller than the type which was originally devised for dogs, a method already reported, experimental hypertension has been produced in monkeys (*Macacus rhesus*). For months before and after the production of bilateral renal ischemia, systolic and diastolic pressures were determined by the Riva Rocci method (the cuff being applied around the lower part of the abdomen) with auscultation over the femoral artery. Systolic and diastolic pressures became greatly elevated following constriction of the renal arteries and a corresponding elevation of "mean blood pressure" was also found by direct puncture of the femoral artery and connection with a mercury manometer. In one monkey the hypertension has now persisted for 13 months. During this entire period the average systolic pressure has been about 150 mm. Hg. higher and the average diastolic pressure about 100 mm. Hg. higher than during the control period. The "mean blood pressure," as determined by puncture of the femoral artery and direct connection with a mercury manometer, also showed an increase of 150 mm. Hg. over the control period.

SPLANCHNIC SECTION IN EXPERIMENTAL HYPERTENSION. Harry Goldblatt and (by invitation) Jerome Gross and Ramon F. Hanzal, Cleveland, Ohio.

Abstract. The purpose of this investigation was to determine whether, in dogs, splanchnic section would prevent the elevation of blood pressure produced by renal ischemia or lower the pressure in dogs with experimental renal hypertension. The lower portion of the dorsal sympathetic chain, including about four

ganglia, and the thoracic portion of the splanchnic nerves were excised by the intrathoracic route. In four normal dogs, after a variable period following this operation, the renal arteries were constricted moderately by the application of an adjustable silver clamp, a method previously described. The systolic pressure rose, as usual, in all four animals and renal function tests showed little or no impairment. In two other dogs, in which the constriction of the arteries was purposely made severe, the blood pressure rose but there was severe impairment of renal function, clinical uremia developed and the animals died in a few days. Excision of the lower dorsal sympathetic ganglia and the thoracic portion of the splanchnics was also performed in four dogs with experimental renal hypertension that had existed for from a few weeks to 4 years. In none of these animals was there any significant change in the blood pressure following the splanchnic section. After excision of the nerves the blood pressure fell temporarily in some of the animals but it soon returned to the original level. It appears, therefore, that in the pathogenesis of experimental renal hypertension due to renal ischemia the vasomotor mechanism of the splanchnic region plays little if any part. These results fail to give experimental support for the operation of splanchnic section that is now being practiced on human beings with hypertension.

Discussion

(Dr. E. T. Bell, Minneapolis.) We are very much indebted to Dr. Goldblatt for this splendid way of producing persistent hypertension in animals. It gives us an opportunity to do a lot of experiments which may help us to understand the fundamental problems of hypertension. It appears from his work that hypertension due to disease of the kidneys is not a reflex phenomenon. A great many investigators have held that obstruction in the renal circulation gives rise to a reflex through the sympathetic which brings about the rise in blood pressure. It is quite clear that this is not the true explanation, and we have to proceed now to find out by what mechanism the blood pressure is increased. I want to ask Dr. Goldblatt if he thinks this obstruction of the arteries might not be analogous to congenital stenosis of the aorta which we see so frequently. A person may live to adult life with a stenosis of the distal part of the arch and show hypertension in the upper part of the body and hypotension in the lower extremities. That goes on to a marked hypertrophy of the heart and death from heart failure or rupture of the aorta in many instances. Is it possible that this obstruction of the renal arteries is the same kind of a process as congenital aortic stenosis? There is another line of study which may be used here: the effect of prolonged hypertension on the smaller arteries throughout the body. I think Dr. Goldblatt should carry on this work and see if he can find arteriolosclerosis in the various organs. One of the young men at Minnesota has used his method, and I have studied the kidneys of animals which have had this type of hypertension for 3 to 4 months, and the arterioles showed no changes in these animals. Possibly the time elapsed is not long enough, or it may possibly be because the pressure is not so elevated in the renal vessels.

(Dr. Goldblatt, closing.) I do not believe that the hypertension of congenital aortic stenosis is directly comparable to the experimental renal type of hypertension, for at least two reasons. (1) In the case of aortic stenosis, the mechanical factor *per se*, by interfering with the onflow of blood, plays a primary part in the pathogenesis of the hypertension; whereas, in renal hypertension, it is the *renal ischemia* produced by the mechanical factor which is of primary importance.

(2) It has been shown by Prinzmetal and Wilson that the vasomotor apparatus plays an important part in the continuance of the state of hypertension associated with aortic stenosis, whereas the experiments reported here indicate that the vasomotor mechanism is of relatively little, if any, importance in experimental renal hypertension.

The possible effect of long continued elevation of blood pressure on a vascular system that was normal prior to the production of the hypertension is, of course, of great interest to us. Some of our animals have had hypertension for about 5 years. We hope as a result of study of such animals to be able to answer Dr. Bell's question about the effect of the hypertension on the vascular system subjected to this elevated pressure. The changes, if any, in the vessels within the kidneys would obviously be due to *hypo*tension and ischemia rather than hypertension and would not be comparable to any changes found in the rest of the vascular apparatus. It is difficult to decide when to terminate the experiments. A negative result, that is, no pathological change in the blood vessels, would be of significance only if the hypertension had existed for a long time. On the contrary, pathological changes found in old animals with experimental hypertension might be attributable to the age factor alone. We shall be obliged to study the possible effect of various periods of hypertension in different animals Changes due to the hypertension ought to progress with the length of time during which the vessels are subjected to the elevated pressure. We are not yet in a position to give an answer to this question. I am very pleased that Dr. Bell is using our method for the production of hypertension.

THE EARLY STAGES OF GLOMERULONEPHRITIS. E. T. Bell, Minneapolis, Minn.

Abstract. A microscopic study of the kidneys was made in 107 cases of death from accidental causes, in 194 cases with death from non-infectious diseases, and in 564 cases of death from various infectious processes.

In the 107 normal kidneys the glomerular epithelial cells definitely outnumbered the endothelial in 84.1 per cent, the endothelial outnumbered the epithelial cells in only 1 instance (0.9 per cent), and the two types of cells were approximately equal in number in 15 per cent. It was concluded that a definite preponderance of endothelial over epithelial cells represents a glomerulitis.

A Grade 1 glomerulitis was found in 14 per cent of non-infectious processes.

In lobar pneumonia glomerulitis was found in only 18.8 per cent, but in the other infectious groups it varied from 37.5 to 78.9 per cent.

The highest incidence of glomerulitis was found in puerperal sepsis (52.4 per cent) and subacute bacterial endocarditis (78.9 per cent).

It is evident that a variety of toxic substances, especially those derived from streptococci, may irritate the glomerular capillaries and produce an increase of endothelial cells.

In a Grade 2 glomerulitis the glomerular capillaries are filled with cells and occasionally a few intracapillary fibers are present. The distinction from clinical glomerulonephritis is somewhat arbitrary.

The glomerulitis is probably due chiefly to endothelial proliferation, but the lodgment of mononuclear leukocytes in the capillaries seems to play a rôle of some importance.

There is no relation between the presence or the amount of albumin in the urine and the degree of endothelial proliferation.

There is no anatomical basis for a diagnosis of focal glomerulonephritis except

in instances of transitory glomerular bleeding, not associated with symptoms of nephritis, and in cases of bacterial endocarditis.

Discussion

(Dr. Paul Klemperer, New York City.) I wonder, Dr. Bell, what you think of the possibilities of the intercapillary glomerulitis — this may be digressing a little bit from your topic — but I wonder how you feel about the intercapillary glomerulitis which was stressed recently by MacCallum and Kimmelstiel. Might not some of the cells which you could not place be fibroblasts?

(Dr. Bell, closing.) I have seen no evidence of intercapillary glomerulitis in this group. I have, however, seen intercapillary changes in some hypertensive forms of kidney disease.

RENAL LESIONS IN EXPERIMENTAL BENCE-JONES PROTEINURIA. H. Edward MacMahon and (by invitation) A. Magnus-Levy, Boston, Mass.

Abstract. There are several interesting questions dealing with the relation between the elimination of Bence-Jones protein and renal injury that are still unsettled. Can Bence-Jones protein pass through a healthy kidney? Where in the kidney is Bence-Jones protein eliminated? Is Bence-Jones protein injurious to the kidney, and if it is, what is the nature of this injury?

Preliminary to our attempt to answer these questions we sought a suitable stain that would enable us to recognize Bence-Jones protein within the tissue. We found that smears made from concentrated solutions of Bence-Jones protein, as well as the pure crystals of Bence-Jones protein isolated from the urine, stained a brilliant red with acid fuchsin and a brilliant yellow with orange G. By combining these stains, as is done in the Mallory aniline blue connective tissue stain, the crystals stain a brilliant orange gold regardless of whether the fixative used was Zenker or formalin solution. It was also observed that the large crystals which are sometimes found lying freely within the substance of myeloma tumors — and regarded as Bence-Jones protein — stained in an identical manner. The abundant precipitate which is so often found within the renal tubules, and the crystals that are occasionally found in the same tubules in cases of Bence-Jones proteinuria, behave with this stain in a similar way. Although this stain, in a chemical sense, is not absolutely specific for Bence-Jones protein, it offers a very good indicator when used with other stains for the presence of this material.

In 1931 we began this study of the renal changes following subcutaneous and intraperitoneal injections of varying quantities of Bence-Jones protein.

Sixty white mice in all were used in this experimental study. The animals were divided into two groups. The animals of Group 1 received a single injection (0.1 gm.) of a 5 per cent solution of Bence-Jones protein. These mice were autopsied at 2, 4, 6, 8, 16 and 24 hour intervals and at intervals of 2, 4, 6 and 8 days. The animals of Group 2 received daily injections during a period varying from 1 to 6 weeks.

Group 1: (Mice of this group had received a single injection.) After *2* and *4 hours* no changes are recognizable in the kidney. As early as *6 hours* the cells of the distal convoluted tubules show at their bases a brilliant orange granulation of their cytoplasm, leaving the cytoplasm adjacent to the lumen unchanged. At this time there is no recognizable change in the glomerulus or

in the vessels, and the tubules are empty. At the end of *8 hours* these same cells of the distal convoluted tubules are shrunken and diffusely and brilliantly stained, making a sharp contrast to all of the cells of the remaining tubules. In the hematoxylin and eosin stained preparations these changes are scarcely recognizable. The glomerulus and vessels are unchanged, and the capsular space of the glomerulus and the lumens of the tubules are empty. After *16 hours* these same cells, singly and in chains, are desquamated. They stain intensely a brilliant orange gold with the Mallory connective tissue stain, and lie freely within the tubule. The lumens of the tubules and the glomerular spaces contain a hyaline substance staining as Bence-Jones protein. At the end of *24 hours* desquamated epithelial cells are still recognizable, and tubules here and there contain a small amount of material resembling Bence-Jones protein. The most striking change at this time is the early sign of regeneration, for mitoses in the epithelial cells are numerous, and the denuded areas within the tubules are being relined. At the end of *1 week* the kidney shows no pathological change and Bence-Jones protein is no longer to be found.

In brief, it does appear from these experiments that Bence-Jones protein may pass through a healthy kidney and that for the most part it is eliminated through the glomerular tuft. Furthermore, it is seen to injure the epithelial cells in a distinct segment of the tubule, leading to death of these cells and ultimate desquamation. The injury is simply one of degeneration, which is quickly followed by a process of regeneration without vascular changes of inflammation. It would seem from the very early appearance of the Bence-Jones protein within the tubular epithelium — before it is found within the glomerular capsule or in the lumen of the tubule — that this protein may be eliminated by the tubular epithelium as well as by the glomerular tuft. Bence-Jones protein may fill the lumens of the tubules without producing any apparent injurious effect on the majority of cells lining the tubules, leading one to believe that as long as it lies outside the cell it is comparatively inocuous, whereas as soon as it is taken up by the cell it leads to regressive changes which may result in necrosis. The lesion that is produced in the kidney following a single subcutaneous injection of this protein is completely reparable.

Group 2: (Mice of this group had received successive daily injections.) At the end of *48 hours* the entire tubular structure of the kidney with the exception of the collecting tubules shows a severe degenerative change. The cells of the proximal convoluted tubules immediately associated with the glomerulus are large, swollen and vacuolated and the cytoplasm is scattered, rather clumped, and granular. These cells do not contain Bence-Jones protein. The next group of cells of the proximal convoluted tubule, as one passes down the nephron, are even more swollen and, in the hematoxylin and eosin stained preparation, show the most extreme form of hyaline droplet degeneration. The cell boundaries are indistinct and the inner cell boundary lining the lumen is incomplete and broken. The cells in places are so swollen and the cell membranes so indistinct that here and there it is impossible to distinguish the lumen or to make out the histological structure of the tubule. For the most part these droplets are largest toward the lumen, whereas the cytoplasm at the base of the cells is dust-like and granular. At times it is impossible to say whether the droplets are within the cytoplasm of the cell or within the lumen of the tubule because of the interruption of the lining cell membrane. In preparations stained with the aniline blue connective tissue stain these droplets stain as Bence-Jones protein. The lumens of the tubules and

the capsular spaces contain a similar material. The glomerular tuft, the blood vessels and the basement membrane of the tubules are unchanged.

After *2 weeks* the histological picture has changed. Droplet degeneration is still present in many of the proximal and distal convoluted tubules. Bence-Jones protein is present within the collecting tubules, and the droplets in the tubules stain, as before, as Bence-Jones protein. The most interesting change at this period is the presence of many tubules lined with young regenerated cells showing here and there mitoses. The cells lining these tubules are flat, rather small and appear undifferentiated. Although Bence-Jones protein is present in the lumens bordered by these cells, their cytoplasm is unchanged. At this period there is very slight and somewhat focal contraction of the cortex. The glomeruli and the blood vessels are still unchanged.

At the end of *4 weeks* and at the end of *6 weeks* changes are first observed in the kidneys grossly. The surface shows a very fine granulation. Microscopically the reconstruction of the cortex is quite obvious and the sclerosing process is more marked. Some of the tubules are dilated, others are collapsed and bordered by an increase in connective tissue. Mitotic figures in the epithelial cells lining the tubules are occasionally seen. Casts of Bence-Jones protein are found within the tubules. The collecting tubules remain unchanged and there is no dilatation of the tubules proximal to the casts within the lumens. Here and there a glomerulus in one of the areas of scarring is small, rather collapsed and free of blood while the capsule and pericapsular tissue are thickened. The blood vessels throughout the kidney show no changes.

In summing up our observations based on this second group of experiments in which Bence-Jones protein was repeatedly injected, it does appear that the kidney suffers a permanent injury, most marked in the tubules with less extensive injury to the glomeruli. The damage is one of degeneration of the epithelial cells and, as in the first group of experiments, the outstanding features of an inflammatory reaction are absent. Degeneration and regeneration with a reconstruction of the kidney take place simultaneously.

Summary: Bence-Jones protein may pass through a healthy mouse kidney. For the most part it is eliminated from the body by way of the glomerular tuft, though there is reason to believe that the tubule may take part in this elimination as well. Bence-Jones protein, when taken into the cytoplasm of the cell in sufficiently large quantities, exerts an injurious effect leading to regressive changes and ultimate necrosis. The change within the kidney affects primarily the tubules and to a lesser degree the glomeruli. The change is essentially one of degeneration. Following a single injection the lesions within the kidney are completely reparable, whereas in the course of successive injections the injury becomes permanent, resulting in sclerosis with contraction. The lesion may be regarded as a form of *nephrosis* and in the late stages where contraction has begun it may be considered a nephrotic form of renal sclerosis.

Realizing that other factors may play an important rôle in the histological changes in the kidney in human cases of chronic Bence-Jones proteinuria, this does not militate against the experimental facts that Bence-Jones protein alone may produce serious renal injury.

Discussion

(Dr. Wiley Davis Forbus, Durham, N. C.) We too have been interested in the excretion of Bence-Jones protein by the human and the animal kidney, and have

reported experiments which are quite similar to those Dr. MacMahon described. We used three types of animals — dogs, rabbits and white mice. In none of the animals were we able to see that the excretion of relatively large quantities of protein over a considerable period of time had any effect whatever on the kidney epithelium. We were unable to produce in any of these three types of animals a lesion which we thought at all comparable to that which is commonly found in the human kidney when relatively large quantities of the protein are excreted. We too were very much interested in the possibilities of identifying Bence-Jones protein by means of characteristic and specific staining reactions, but we never succeeded in convincing ourselves that this could be done by means of the numerous stains which we used. We followed our animals for a considerable period of time. We also controlled the lesions in the kidneys by preliminary removal of kidney tissue. We did not, as Dr. MacMahon did, examine the kidneys of our animals shortly after the administration of protein, but our animals were excreting Bence-Jones protein when they were sacrificed. We have no information as to what might happen within a short time, let us say a matter of a few hours after the substance is given. It seems rather strange that injuries which are so pronounced as those described by Dr. MacMahon did not leave some permanent effect in the kidney in our animals, assuming that the protein had produced such injury immediately following the administration.

(Dr. J. Furth, New York City.) I wonder if Bence-Jones protein is antigenic and, if so, whether it would not be expected that in a heterologous species it would provoke a reaction that would not occur in a homologous species.

(Dr. MacMahon, closing.) I wish to thank Dr. Forbus for discussing this paper in such detail. I am very familiar with his paper which appeared only a few months ago. I wonder if by chance the apparently contradictory results of his experiments could be explained in part either by a difference in quantity of the material injected or by a variation in the concentration of the material injected. In our experiments minute quantities failed to produce recognizable changes, and equally important is the fact that when concentrated emulsions of Bence-Jones protein were injected subcutaneously it remained localized in the tissues for some time. The protein set up an inflammatory reaction about the deposit, but produced no significant renal changes. In contrast with this finding, we repeatedly observed that when a moderate amount of this protein was injected in dilute solution subcutaneously it appeared very soon in the urine and produced striking regressive changes in the tubular epithelium and less marked changes in the glomeruli.

In regard to the specificity of the stains, I do not believe that from a chemical standpoint the Mallory connective tissue stain is specific for Bence-Jones protein. Serum globulin and fibrinogen, for example, stain in a somewhat similar way, but it does appear that if used with other stains the Mallory connective tissue stain offers the best method of demonstrating this protein within the tissues.

This protein is antigenic. In our control experiments other human serum proteins were used without producing similar reactions.

AMYLOIDOSIS WITH UNUSUAL DISTRIBUTION AND BENCE-JONES PROTEIN. H. E. Robertson and (by invitation) L. A. Brunsting, Rochester, Minn.

Abstract. In the case of a man 44 years of age, who was otherwise in good health, muscular pains and weakness, particularly in the shoulder girdle, and yellowish nodules on the skin, especially of the eyelids and face, developed over a period of

2 years. The nodules were deeply colored by Congo red. The patient's urine contained Bence-Jones protein. No traces of myeloma could be found. At autopsy, in addition to the corium, amyloid was found in the tongue, walls of the esophagus, stomach, small intestine, colon and bladder. Mere traces were present in the kidneys, liver and spleen. A softened marrow in a vertebra was the only sign of a lesion to be found in the bones: this marrow contained a large increase in plasma cells, suggesting a cryptic origin for the proteinuria.

Discussion

(Dr. Shields Warren, Boston.) I should like to ask Dr. Robertson whether the presenting symptoms in this case were the cutaneous nodules, or the involvement of the tongue. In 2 somewhat similar cases we have had, not so carefully studied unfortunately, we made the diagnosis in the 2nd case from the involvement of the tongue. The patients in both instances complained that their tongues were too large for their mouths, they had difficulty in swallowing, and their tongues had the characteristic rubbery feeling of the amyloid spleen or liver. I wonder whether in view of the amount of amyloidosis in the tongue in Dr. Robertson's case the same finding occurred.

(Dr. H. E. MacMahon, Boston.) One of the most striking features of amyloidosis associated with multiple myeloma is the localization in which the amyloid is deposited. In contrast with the usual forms of amyloidosis associated with chronic suppuration in which the amyloid is confined primarily to the liver, kidney, spleen, adrenal and intestine, one finds it deposited in large masses in the bone marrow, in muscles, in cartilage, in connective tissue and even in fat tissue in cases of multiple myeloma associated with Bence-Jones proteinuria. The distribution of the amyloid in the case reported by Dr. Robertson follows more closely to the rule than to the exception for this group of amyloid diseases. In our experimental work in which Bence-Jones protein was injected over a long period of time in animals, we had hoped to find some trace of amyloid in the tissues. In no case, however, could we demonstrate experimentally the conversion of Bence-Jones protein into amyloid material. It is of interest that both Bence-Jones protein and amyloid are stainable with Congo red and if this were the only stain used for the demonstration of amyloid the results could be open to criticism, as this dye stains other forms of hyaline and colloid material as well.

(Dr. Robertson, closing.) The symptoms were not referable to the tongue in this case. The man complained of pain, particularly along the shoulder and hip girdles, and he also exhibited weakness which almost reached a myasthenia gravis type and probably resulted from an infiltration of the muscular bundles.

"Fibrinoid" Lesions in Atherosclerosis and Syphilitic Aortitis and their Relation to Thrombus Formation in the Aorta and Coronary Arteries. Eugene Clark (by invitation), Irving Graef, and (by invitation) Herbert Chasis, New York City.

Abstract. The term "fibrinoid" has been employed by many in designation of a substance encountered in the walls of affected arteries, which in its tinctorial behavior resembles fibrin. Mallory has described the occurrence of fibrin-like material in the intimal plaques of atherosclerosis and syphilitic aortitis, and has expressed the belief that this substance represents organizing fibrin. Recently, however, a different interpretation of the nature and source of this material has appeared. Yager believes that the fibrin staining material in the intimal aortic

plaques of atherosclerosis and syphilitic aortitis represents degenerated or necrotic fibrous tissue, and Leary has expressed a similar opinion of the fibrin staining material in the fibrous regions of the intimal plaques in coronary thrombosis. The subject has gained new and wider importance by their assertion that thrombosis may be brought about in such vessels when fibrinoid necrosis extends to the surface of the plaques.

This report is based on the study of autopsy material from the Bellevue Hospital. It comprises an examination of the plaques of 40 atherosclerotic and 38 syphilitic aortas in which thrombosis was not grossly visible. Proceeding from these observations we have considered the lesion in the intimal plaques of 9 parietal aortic thrombi and of 11 instances of coronary thrombosis, the latter studied by serial sections. In those instances where material resembling fibrin appeared in the intimal plaque, successive sections were stained by hematoxylin and eosin, Weigert's elastic tissue stain, Van Gieson's, Mallory's phosphotungstic acid hematoxylin, Gram-Weigert, the silver impregnation method of Foot and Foot, and the benzidine stain for hemoglobin. Though the tinctorial reaction of the material to gentian violet was not constant, the fibrinous material of the thrombi exhibited a similar inconstant behavior. With the other methods employed no essential difference in staining reaction could be discerned between the material within the plaque and the fibrinous component of thrombi.

In 17 of the 38 specimens of syphilitic aortitis, fibrin staining material, generally of homogeneous character, was found in the fibrous regions of the intimal plaques. In the majority of instances the fibrin staining material appeared as horizontal or oblique bands on the surface and within the superficial fibrous regions of the plaques. Young fibroblasts and argyrophilic fibrils were frequently observed in intimate relation to the fibrin staining masses; regressive or inflammatory changes were absent. In the fibrous regions of the intimal plaques of atherosclerotic aortas similar lesions were found in 15 of the 40 specimens studied. They were found in fibrous plaques, in the fibrous covering of nonulcerated atheromas, and in the fibrous regions of ulcerated plaques. In parietal aortic thrombi, fresh thrombi were frequently deposited on a layer of dense homogeneous fibrin staining material, which was continuous with oblique and horizontal bands of similar tinctorial properties in the superficial fibrous regions of the underlying plaque. Similar findings were encountered in thrombosed coronary arteries, though more commonly in such vessels the fibrin staining material in the plaque was confined within lipoid zones.

The gradual transition from masses which are clearly surface deposits to those covered by endothelium and collagenous tissue with young fibroblasts yields strong support for the belief, first expressed by Mallory, that these fibrin staining bands in fibrous plaques represent repeated surface deposits that have undergone partial organization. In other instances, of less common occurrence, the findings suggest that a break in the collagenous covering of an atheroma has permitted penetration and dissection of the plaque by blood elements.

If by thrombosis we mean the deposition of blood elements on the vessel wall, then these fibrin staining bands in most instances represent the remnants of organizing parietal thrombi, which have become covered by connective tissue. On such laminated, condensed, and partially organized surface deposits a fresh thrombus of formed elements and orthodox configuration frequently supervenes.

In the coronary arteries, in some instances, thrombosis represents a slowly progressive process in which the repeated parietal deposition of blood elements

is followed by partial organization which may lead to an increase in the size of the plaque and a progressive stenosis of the vessel. Such organizing parietal thrombi may form the base on which a fresh occluding thrombus is deposited. In other instances coronary thrombosis occurs suddenly, an occluding thrombus forming on an ulcerated atheromatous plaque into which penetration of blood elements has occurred.

Finally, we could find no evidence to support the view that the deposits of fibrin staining material in the intimal plaques of atherosclerosis or syphilitic aortitis represent altered collagenous fibers. The tinctorial properties of this so-called fibrinoid material are attributable to its fibrinous component, though other blood elements which have lost their recognizable character may possibly share in its composition.

Discussion

(Dr. Ralph D. Lillie, Washington, D. C.) May I ask whether in the course of these studies the relation of these fibrinous masses to the internal elastic membrane satisfactorily stained was demonstrated. I would feel more convinced if that technical procedure had been used, although the presentation sounds very convincing in any case.

(Dr. Clark, closing.) In answer to Dr. Lillie's question, successive sections were stained by the methods reported, which included the Weigert elastic tissue stain combined with the Van Gieson. The elastica was clearly outlined in these sections and separated the thick intimal plaque from the media. These fibrinous masses were always found in the superficial regions of the plaque, and they appeared lemon yellow with this stain.

ARTERIOSCLEROSIS: A COMPARISON OF THE PATHOLOGY AND CONSEQUENCES IN THE NEGRO AND WHITE RACES. G. H. Hansmann, Milwaukee, Wis., and (by invitation) J. R. Schenken, New Orleans, La.

Abstract. A review of the postmortem material at the Gallinger Municipal Hospital, Washington, D. C., and the Charity Hospital, New Orleans, substantiated the observations of a number of Southern and Northern clinicians that coronary thrombosis secondary to coronary arteriosclerosis was rare in the negro race. Gross and microscopic examination of the aortas and coronary vessels obtained from negroes with arteriosclerosis, as compared to material from the white races, revealed deeper lying atheromatous plaques with greater subendothelial connective tissue proliferation, a decreased incidence of atheromatous ulcers and a greater involvement of the media by the atheromatous process, resulting in localized dilatation of the vessels which appeared to aid in the maintenance of adequate patency. These anatomical differences are offered as the chief explanation for the low incidence of coronary thrombosis in the negro. It is suggested that the atheromatous material acts as a mild irritant and that the subendothelial connective tissue reacts more vigorously in the negro than in the white, similar, perhaps, to the marked connective tissue reaction so commonly seen in the negro following skin and subcutaneous tissue injuries.

THE RELATION OF PERICARDIAL ADHESIONS TO CARDIAC HYPERTROPHY.
R. M. Hosler (by invitation) and Francis Bayless, Cleveland, Ohio.

Abstract. It is generally considered that fibrous pericardial adhesions may cause cardiac hypertrophy. This report includes a study of 281 cases of adults with adherent pericardium found in a survey of 15,000 autopsies in the Institute of Pathology of Western Reserve University (1898–1936) and the Cleveland City Hospital (1915–1936). There were 182 males and 99 females.

The autopsy protocols were abstracted with verbatim descriptions and measurements of the hearts, the condition of the kidneys and lungs, the appearance of the arterioles, the weight and stature of the patients, the blood pressure, and other points of importance in assaying the relation between the weight of the heart and the type and distribution of the accompanying pericardial adhesions. In some instances the clinical record was also reviewed.

For comparison, normal heart weight figures for adult males and females were taken from the tables published by Roessle and Roulet (439 males and 115 females) and by Smith (534 males and 320 females). Curves were drawn to show these maximum and minimum normal variations for the different age periods in the two sexes. On these curves were plotted the weights for the 112 non-hypertrophic hearts found in the series (87 males and 25 females). There were no significant variations from the normal.

In the series there were also 95 males and 74 females with genuine cardiac hypertrophy. The mean heart weight for the males was 899.2 gm., for the females 548.9 gm. In every case there was an adequate explanation for the cardiac hypertrophy, other than pericardial adhesions, such as inflammatory heart disease, hypertensive heart disease, severe coronary arteriosclerosis and myocardial damage, extensive pulmonary disease accompanied by right heart hypertrophy, and various combinations of cardiac diseases.

The type and distribution of the pericardial adhesions were classified as (1) local, (2) subtotal, and (3) complete obliteration of the sac. The cases were also analyzed to show the situation and severity of extrapericardial, or pericardiomediastinal, adhesions. The hypertrophic and non-hypertrophic hearts were then compared on this basis. There were 17.8 per cent hypertrophic hearts and 17.8 per cent non-hypertrophic hearts with Type I adhesions; 5.4 per cent hypertrophic hearts and 11.7 per cent non-hypertrophic hearts with Type II adhesions; and 76.8 per cent hypertrophic hearts and 71.5 per cent non-hypertrophic hearts with Type III adhesions. Calcified pericardium occurred with both hypertrophic and non-hypertrophic hearts.

Summary: The results of the study establish that cardiac hypertrophy is not related to pericardial adhesions. When the heart is hypertrophic an adequate explanation, other than pericardial adhesions, is always found.

Discussion

(Dr. Howard T. Karsner, Cleveland.) It is to be more strongly emphasized than the time limit of Dr. Bayless' presentation permitted that, in spite of the widespread teaching that pericardial adhesion causes cardiac hypertrophy, such teaching is no longer valid. Pericardial adhesion can now be removed from the list of causes of hypertrophy of the heart. Other studies of the condition have occasionally led to the same conclusion but they were not based on a number of cases as large as that covered in this report.

THE DEVELOPMENT OF AN ANTIVIRAL PRINCIPLE IN LYMPH NODES. Philip D. McMaster and (by invitation) John G. Kidd, New York City.

Abstract. Rabbits were inoculated intradermally in one ear with a standard amount of vaccine virus and in the other with typhoid bacterin. In half the instances the ears were amputated an hour later. After later intervals of from 2 hours to 15 days the cervical lymph nodes of both sides, inguinal nodes, bone marrow and spleen were removed and extracts of them were separately inoculated into normal rabbits (as was serum procured at the same time) to test for their content of virus. In addition, neutralization tests were set up to learn whether or not the materials possessed antiviral properties.

By the 2nd or 3rd day an increase in virus was demonstrable in the extracts of the nodes on the virus-injected side. A rapid decline in the amount took place thereafter and by the 7th to the 9th days the extracts failed to produce lesions. At no time did the serum or extracts from other nodes or organs give evidence of virus.

The neutralization tests showed an early appearance of neutralizing ability in the extracts of the nodes from the virus-injected side. By the 4th day 10 per cent extracts of these nodes inactivated fresh vaccine virus diluted to 10^{-5} and 10^{-6} to a greater degree than did whole serum. Serum of the same animal, similarly diluted, possessed no neutralizing power, nor did the extracts of inflamed cervical lymph nodes from the other side, nor the extracts of spleen or bone marrow or of either ear. Virus was found only on the virus-injected side, in the ears and cervical lymph nodes. The negative findings in the inflamed nodes on the side injected with typhoid bacterin served to control the possibility that a concentration of antiviral bodies formed elsewhere in the body and circulating in the blood had taken place in the nodes of the virus-injected side.

Experiments were done to test whether a neutralizing principle might be formed in the tissue of the injected ear, and reach the nodes by drainage. Extracts of the tissues of both ears as well as of the cervical nodes of both sides 4 days after inoculation were separately filtered through Seitz pads and tested. The filtration separated virus from antiviral principle in the extract of the cervical nodes from the virus-injected side, in which both were present together, only the antiviral properties passing the filters. The ear extract totally lacked antiviral power and so too did that of the glands on the control side, whereas that of the glands on the injected side possessed it. Only virus was present in the ear extract and only in that from the virus-injected side.

The results from day to day with serum and extracts of organs other than the lymph glands from the virus-injected side indicate that the latter were a major source of the neutralizing power of the serum.

Discussion

(Dr. Paul R. Cannon, Chicago.) I have been particularly interested in this paper because for a number of years we have been interested in the problem of the local formation of antibodies. Our greatest difficulty has been in demonstrating antibodies before their appearance in the blood serum. I should like to ask Dr. McMaster what was the earliest time period at which antiviral substances were detected in the lymph nodes. It seems to me that the evidence here submitted is more conclusive than in most of the experiments that have been re-

ported for bacterial antigens and that these experiments are quite convincing for the demonstration of the local formation of antibodies.

(Dr. Jules Freund, New York City.) Dr. Rich, I believe, made some very interesting observations on the effect of immunization on lymphocytes. I think your study on the local formation of antibodies in lymph nodes offers a unique opportunity to investigate the possible rôle of lymphocytes in antibody formation. Were histological studies made which might reveal morphological changes in lymphocytes? I may add to this a statement as to a local formation of antibodies at the site of the injection of the skin. I wish to say that in our studies on immunization with heat-killed typhoid bacilli no local antibody formation was found in the skin at the site of injection. I am mentioning this, for there are statements in the literature that there is such a local antibody formation in the skin.

(Dr. Arthur W. Wright, Albany.) I should like to ask Dr. McMaster if by any chance he tried neutralization experiments using lymph node extracts from animals that had not been inoculated with virus but in which hyperplasia and hypertrophy of the lymph nodes had been produced by other means, in other words — extracts of hyperplastic lymph nodes of animals that had not had virus at all.

(Dr. McMaster, closing.) In reply to Dr. Cannon's question, we have not been able to find antibodies in the lymph node extracts before they appeared in the serum. We have always found them present in both, and all I can say is that they are stronger in the lymph node extracts than in the serum a few days after the animal has been injected with virus in one ear — for example, on the 4th day. By the 7th day they are as strong or much stronger in the serum than in the node extract. On the 3rd day we found some degree of neutralization by the lymph node extracts, but I do not believe that neutralization tests are good enough to distinguish between the small amount of antiviral principle found in the node extracts and that found in the serum at that time.

In reply to Dr. Freund, we have not made histological studies as yet. We have saved our sections, but so far I have nothing to report. We have not worked with the skin at all, except as it entered into the filtrates of ear extracts, which included the skin and all other tissues above the cartilage, and, if there were vaccinia lesions present, it included those too and any other inflammatory material from the ear. We worked with the extracts and filtrates of all these tissues, not with skin alone.

Concerning the question of Dr. Wright's about the possible presence of neutralizing principles in lymph nodes of animals suffering from infections other than vaccinia, we have used for controls those rabbits in which we injected typhoid vaccine in the other ear. Before we began these experiments we obtained lymph nodes from a series of animals and, by mixing extracts of these nodes with known amounts of virus, tested the effect of the extracts on the ability of the virus to produce lesions. In these preliminary experiments we also used extracts of enlarged and inflamed lymph nodes from some rabbits which had been injected in the ears with typhoid vaccine. All gave negative findings in neutralization tests.

THE MORPHOLOGY AND DISTRIBUTION OF THE INCLUSION BODIES OF THE SUBMAXILLARY GLAND VIRUS IN ADULT AND FETAL GUINEA PIGS. Floyd S. Markham (by invitation), Columbus, Ohio.

Abstract. Evidence based on a study of both fresh and fixed tissues suggests that the inclusions associated with the submaxillary gland virus of guinea pigs consist of a chromophile matrix in which are embedded corpuscles resembling those seen in certain other virus inclusions.

These corpuscles may be stained in Zenker-fixed tissues by Harris' hematoxylin differentiated in a half saturated solution of picric acid.

In tissues prepared by Gersh's modification of the Altmann technique, both the intranuclear and cytoplasmic inclusions give a positive reaction to the Feulgen stain for thymonucleic acid.

The inclusions in intracerebrally inoculated young guinea pigs are confined to the cells of the meningeal exudate, and inclusion-laden cells are not found in the circulating blood. Intracerebral inoculation of virus emulsions into the guinea pig fetus *in utero* is frequently followed by invasion of the blood stream by inclusion-laden mononuclear cells and the appearance of foci in most of the extracranial tissues, especially the placental mesenchyme.

Discussion

(Dr. S. Burt Wolbach, Boston.) This paper is personally very interesting because we, in Boston, encounter identical inclusions so frequently in the salivary glands and other organs of infants, newborn and stillborn. Perhaps you will recall that many years ago Dr. Allan Smith of Philadelphia regarded these inclusions as protozoan in character and submitted the preparations to Hertwig who pronounced them as cells, not of mammalian origin. Another interesting thing is that they are found so frequently in rodents. I should like to ask Dr. Markham the nature of the material which he used for inoculation — was it cell débris or filtrates? You may recall that Dr. Farber and I made a very careful study in the endeavor to correlate the presence of these inclusions with any one type of disease, but we found them distributed regardless of the nature of the illness exhibited or the cause of death. Needless to say, our opinion was strongly against these inclusions being the cause of pertussis, a theory which has been advanced.

(Dr. Markham.) In answer to your question about the nature of the inoculum, both types of inoculum have been used, that is, filtrates of emulsions of submaxillary gland and simple sedimented emulsions.

(Dr. Wolbach.) You did not employ human material?

(Dr. Markham, closing.) No, so far as the reports go I know of only one other instance, I believe that of Kuttner, who has reported attempts to infect rodents with emulsions of human material.

ON THE PATHOGENESIS AND IMMUNOLOGY OF POLIOMYELITIS. N. Paul Hudson and (by invitation) Edwin H. Lennette and Francis B. Gordon, Columbus, Ohio.

Abstract. Experimental evidence is presented to indicate that the olfactory nerve endings in the nasal mucosa are the portal of entry of the poliomyelitis virus. Five monkeys with sectioned olfactory tracts were not infected by intranasal virus, and later they did not show symptoms of poliomyelitis after intra-

venous inoculation of virus lethal to four of five controls. The possibility of infection here resulting from intravascular virus being exuded onto the nasal mucosa and thence by the olfactory tracts is supported by our finding the virus in nasopharyngeal washings of other monkeys after intravenous injections. No evidence of infection was found in four monkeys given huge and repeated amounts of virus-cord into isolated intestinal loops with external openings. Another possible route of infection is by the blood stream to the central nervous system. Against this as a natural route is the fact that only very large intravascular injections are infective, and furthermore the existence of a blood-CNS barrier was demonstrated by experimental infections resulting from small intravenous doses of virus at the same time that injections of sterile starch were made into the brain. From these experiments it appears more clear that the virus of poliomyelitis under natural conditions enters the central nervous system through the exposed hairs of the unmedullated nerve fibers of the olfactory tracts.

Evidence furnished by other workers shows the intraneural migration of the virus to the cord. We propose that the same occurs even without signs of infection and that there is an overflow of virus into extraneural tissue with the resulting formation of antibodies. In support of this view, the virus has been recovered by us from perfused spleens of two monkeys during the acute attack after cerebral inoculation. We consider on the basis of our experimental studies that the neutralization test in man and monkeys is a specific antigen-antibody reaction, and under natural conditions an evidence of extraneural stimulation of antibody-forming tissues after neural migration of virus. Under conditions of artificial immunization in the monkey, specific antibodies may be induced, but measures to establish an effective immunity against experimental infection have been found necessarily so extreme that they are correspondingly unsafe for human application. Furthermore, the presence of serum antibodies, as we have experimentally determined, is not a certain indication of effective resistance to virus administered by the natural intranasal route.

Discussion

(Dr. Jules Freund, New York City.) I think that in judging the possible rôle of antibodies in the resistance to poliomyelitis it is desirable to consider not only the presence or absence of the neutralizing antibody in the blood, but also the titers of the serums. It is possible that in poliomyelitis the antibody content of the central nervous system is independent of that of the blood, but with agglutinins against typhoid bacilli there is a numerical relation between the antibody content of the blood serum and that of the spinal cord, the ratio being 100 to 0.8 (*J. Exper. Med.*, 1930, **51**, 889).

(Dr. Hudson, closing.) In reply to Dr. Freund, we are not able to answer Dr. Freund's question at the moment. The work on the quantitative side is being done. We feel, however, that the neutralization test is a rather crude test and, whereas some information may be given by the titration of antibodies, the variations that have occurred are too frequent for us to draw any fine and quantitative line. We realize that we can immunize against poliomyelitis experimentally, and presumably what Dr. Freund has indicated follows. However, what we are trying to point out here is that when we use a method which is close to the fatal line, a severe method, in the presence of antibodies, still such a condition is not effective against the intranasal administration of the virus. This point is well taken and is being pursued.

ON THE PROBABLE NATURE OF THE INFECTIOUS AGENT OF TRACHOMA. L. A. Julianelle, St. Louis, Mo.

Abstract. The studies published from this laboratory have already established trachoma as an infectious disease, transmissible to monkeys and apes. Independent of faulty or deficient diet, it was shown that neither bacteria cultivated from the conjunctival sac, nor filtrates of infected tissues obtained by filtration with Berkefeld V filters can be assumed as logical factors in the infection. In developing further the concept of infectivity of trachoma, it was found that active tissues retain their original potency after passage through rabbit or guinea pig testicle. Tissues originally non-infectious do not acquire infective capacity by testicular passage. By this technic, the infectious agent apparently does not multiply but is merely preserved over a period of 2 weeks or more, which suffices for purification of the original material from the extraneous bacteria usually present in trachomatous eyes. Sections and impression smears of active testicles fail to show any definite histological change, and no structures, including the so-called initial and elementary bodies of the human, have ever been observed. It appears, therefore, that the infectious agent of trachoma is a virus.

Attempts to cultivate the virus from human, monkey and rabbit tissues have thus far been unsuccessful. Inoculation of active material in minced chick embryo, in fertile eggs, and in plasma clots containing epithelial cells has been tried repeatedly without success. Inoculums of conjunctival cells direct from infectious patients in their own plasma clots have yielded distinct growth of epithelial cells, but the virus not only did not grow but even failed to survive the conditions of tissue culture. Experiments are still under study on cultivation of the virus, so that it is not desirable to make any conclusions.

Recognizing the difficulty of generalization, since the variation in infectivity of tissues and susceptibility of animals is great, it seems fair to offer the following characteristics of the trachoma virus. It is inactivated by heating at 45 to 50° C. within 15 minutes; is rendered non-infectious by the action of bile which causes lysis of epithelial cells and inclusions; does not survive exposure to dilutions of 1:100,000 gentian violet, 0.25 per cent phenol, 2 per cent silver nitrate, 4 per cent cocaine, and in about half the tissues tested, even 2 per cent cocaine. Preservation of the virus in 50 per cent glycerine, still under study, indicates that glycerine does not maintain the virus any longer than storage without preservative at icebox temperature. Ordinary preservation at this temperature varies with individual tissues up to a week or more; at room temperature the virus may survive up to 24 hours; at body temperature survival is a matter of hours only. While singly none of the characteristics depicted helps to identify the agent, collectively they suggest, as brought out by the testicular experiment, that the infectious agent of trachoma is a virus. The part played by the epithelial cell inclusion will have to await future investigation for possible clarification.

Discussion

(Dr. Edward C. Rosenow, Rochester, Minn.) I should like to ask whether the material inoculated into the rabbits from which the virus was derived does or does not contain bacteria, especially *B. granulosis*, as described by Noguchi.

(Dr. Julianelle, closing.) The material which comes from the eyes in trachoma is always contaminated with extraneous bacteria, and those bacteria are inoculated with the tissues in the rabbits. The testicular tissue after a couple of weeks

is bacteriologically sterile. In some instances *B. granulosis* described by Noguchi was present, but this organism does not cause any reaction in monkeys, so there is no particular point in discussing it.

THE PATHOLOGY OF THREE CASES OF GLYCOGEN STORAGE DISEASE ILLUSTRATING SOME UNUSUAL FEATURES. F. W. Wiglesworth (by invitation), Montreal, Canada.

Abstract. The 3 cases to be presented were observed within a period of 1 year at the Children's Memorial Hospital, Montreal.

Case 1. This child, a male, aged 3 years, presented the typical picture of von Gierke's disease, both clinically and biochemically. Biopsy of the liver was done and estimation of the glycogen showed the liver to contain 18 per cent. Sections demonstrated an early portal cirrhosis with greatly swollen liver cells packed with glycogen. The child died 3 months later of pneumonia.

At autopsy the liver weighed 2155 gm. Chemical analysis showed the liver to contain 11 per cent glycogen and the sections revealed a corresponding decrease as compared to the biopsy specimen. The polymorphonuclears in the lung were laden with glycogen, while the other organs contained only traces of it.

Case 2. A male infant, aged 2 months, a younger brother of the patient in Case 1, died of meningococcal meningitis 24 hours after admission to the hospital. Sections revealed a moderate amount of glycogen in the liver cells, although autopsy was performed 10 hours after death. This case points to a definite familial tendency of the disease.

Case 3. A female infant, aged 11 months, who clinically did not suggest glycogen storage disease, was diagnosed as suffering from cirrhosis of the liver. The blood sugar was 42 mg. per cent — a finding that might have led to the proper diagnosis. Autopsy showed a liver weighing twice the normal weight with gross changes suggesting cirrhosis. Histologically there was a well developed portal cirrhosis with large liver cells containing glycogen, although the tissue had been fixed in watery fluids. The spleen showed moderate amounts of glycogen in the reticular cells.

The findings in these 3 cases point to the following conclusions:

1. That the disease may lead to cirrhosis.
2. That there is a familial tendency.
3. That the condition is present from an early age and before signs or symptoms can be noted.
4. That the reticular cells of the spleen may take up glycogen.
5. That possibly the glycogen is mobilized during an acute disease such as pneumonia.

Discussion

(Dr. Edwin F. Hirsch, Chicago.) Did any of these patients have symptoms of diabetes? The clinicians of St. Luke's Hospital, Chicago, have a boy 17 years of age, apparently afflicted with this disease, but also showing symptoms of diabetes. The results of the adrenalin and glucose tolerance tests were those of a patient with diabetes. A small piece of liver tissue removed for examination showed the changes that occur with glycogen storage.

(Dr. David Perla, New York City.) Did you test for glycogen in organs other than the liver? It is a very interesting thing that in experimental infections of many types the normal storage of glycogen very rapidly diminishes, and in these

animals it is almost impossible to determine the presence of any glycogen at all in the liver, whereas the normal animal may contain large amounts. Have you any idea as to the nature of the disturbance in this disease?

(Dr. H. Edward MacMahon, Boston.) Dr. Wiglesworth has pointed out that the polymorphonuclear leukocytes in the inflammatory exudate in the lungs of one of his patients showing this interesting disease contained glycogen. In making routine studies of pneumonic exudates it is quite common to find a moderate amount of glycogen in these cells. I should be interested in knowing if the amount of glycogen in the polymorphonuclear leukocytes was increased in this disease, and if from a diagnostic standpoint the presence of glycogen in the leukocytes of the circulating blood is of value.

(Dr. Wiglesworth, closing.) I may say that in the first case which I showed, and which we followed for 1½ years before death, there was no evidence of diabetes and no sugar in the urine at any time. The other cases were followed for only brief periods, but they had no sugar in the urine.

As to the presence of glycogen in the other organs, the muscle at biopsy showed some. I cannot say whether there was an excess or not. The rest of the organs at autopsy showed no glycogen in any amount. Chemical analysis of the brain, spleen and kidney showed traces of glycogen.

As to the nature of this condition, I am afraid I do not know. The condition is thought to be a continuation of the fetal metabolism. Apparently in the fetus the glycogen is extremely stable, just as it is in this condition.

Concerning the exudate in the lungs, polymorphonuclears were examined for glycogen during life and there was no glycogen in them at that time, when the child was in perfect health; but, at autopsy, sections of the various organs showed polymorphonuclears containing glycogen in the blood vessels.

PATHOLOGY OF THE URACHUS. James E. Davis and (by invitation) George Hammond and L. Yglesias, Detroit and Ann Arbor, Mich.

Abstract. The pathology of the urachus is closely linked with and influenced by the developmental history of the urinary bladder and the midportion of the anterior abdominal wall. The completed transition of the cloaca and allantois, with the regression of the umbilical arteries and apical part of the bladder wall to ligamentous supports, is also of great importance.

Obliteration of the urachal canal by atrophy of its epithelial lining depends on elongation of the body trunk, thereby separating widely the umbilicus and the bladder apex, and also on lateral tension of the two strongly retrogressing umbilical arteries. These, together with abandoned function, should normally convert urachal structures to a ligamentous cord with effectual prevention of fractional function of its epithelial cells. The degree of incompleteness in the foregoing changes determines urachal pathology.

The literature on the urachus should be extended as an enrichment to our knowledge in urology.

Sixty dissections of the urachus in fetuses and newborn infants have been made by the authors and interesting anatomical observations have been recorded pertaining to:

1. Complete covering of the urachus by peritoneum in all cases.
2. Closure of the bladder end of the urachus by Wutz's ligament in all normal formations.
3. The obliterated umbilical arteries and body growth elongation which cause striking variations in the ultimate intactness of the urachus even at birth.

4. A firm distinct fibrous sheath, separable from the peritoneum and anterior to it, which is found between the umbilical arteries and folded about them.

5. Variations in the urachus which were noteworthy in umbilical hernias and ectopias.

Four new cases of carcinoma are reported, all of which appear adequately proved, with 1 more, lacking in diagnostic data.

Three additional unproved tumor cases are cited.

Three differential diagnostic cases are mentioned.

Discussion

(Dr. Theodore J. Curphey, Westbury, N. Y.) I should like to ask whether Dr. Davis draws any distinction between the fibroadenomas, or adenomyomas, and endometriosis of the umbilicus, which I think Dr. Weller described some time ago. Are they different lesions?

(Dr. Davis, closing.) In Begg's contribution to the tumors of the urachus (*Brit. J. Surg.*, 1931, **18**, 422) displacements of the epithelial lining into the surrounding connective tissues are well pictured. This epithelium is of transitional type with here and there goblet cells, thus differing from the endometrial epithelial pattern. In the adenomyomatous formations or in endometriosis the taller endometrial cells are surrounded by extravasations of red blood cells at each menstrual period. In all male subjects endometriosis and adenomyosis would at once be excluded. The lesions of urachal origin are different lesions.

RETINAL TUMORS IN TUBEROUS SCLEROSIS. B. Earle Clarke, Providence, R. I.

Abstract. Involvement of the retina in cases of tuberous sclerosis escaped notice until 1921 when Van der Hoeve (*Arch. f. Ophth.*, 1921, **125**, 880), a Dutch ophthalmologist, published a clinical report describing 6 cases in which tumors of the retina were observed through the ophthalmoscope. Since then a number of clinical reports have appeared, indicating that such retinal tumors are not uncommon and that disturbance of vision and the ophthalmoscopic examination may give the first clue to the disease.

Two years later (1923), Van der Hoeve (*Arch. f. Ophth.*, 1923, **111**, 1) published a pathological study of the retinal tumors from 2 of his 6 cases. From 1 he had only one eye which was enucleated because of a suspicion of malignant change. In this eye there was a large tumor of the optic disc, which he regarded as the primary growth, and several smaller secondary tumors scattered over the retina. In the other case, both eyes were removed at autopsy. There were no tumors of the papillae but several retinal tumors in each eye. The histology in both cases was essentially alike. He described the tumors as being made up of nerve fibers and a peculiar kind of cell. The fibers, he believed, came from the nerve fiber layer and extended through holes in the membrana limitans interna into the overlying tumor. The cells are described as having much cytoplasm which, in places, fused with that of neighboring cells to form a syncytium. The nucleus was usually large with a prominent nucleolus. In the papillary tumor there was a large incrustation. In all the tumors there were spaces without any special lining filled with blood and serum. Blood vessels were sparse. The tumors appeared to originate in the nerve fiber layer and frequently extended to involve the ganglion cell layer, but seldom any others. On the surface of the large tumor of the optic disc were button-like projections which, he believed, became pinched off to float in the humor and then became implanted

elsewhere on the retina. Indeed, he actually observed this occur through the ophthalmoscope.

I have been able to find only three additional pathological studies of these tumors — all in German. In 1925 Schob (*Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1925, **95**, 588) published his findings in the eyes of a 6 year old child who died of tuberous sclerosis. There were no tumors of the disc. Grossly, small whitish, slightly raised areas could be seen on both retinas. Microscopically some were flat and some sharply raised. He described buds and daughter buds with constricted necks sitting on the surface of the larger tumors. In his case the tumors were limited to the nerve fiber layer. He, too, described fibers but concluded that they were definitely glial fibers and not nerve fibers. He also described a syncytium and giant cells with two or three nuclei. The nuclei were large and vesicular with little chromatin and with one to three nucleoli. They were oval, long, curved and sometimes dumb-bell shaped.

In 1930 Feriz (*Virchows Arch. f. path. Anat.*, 1930, **278**, 690) reported a histological study of a solitary tumor of the left eye which did not involve the disc. This tumor involved the nerve fiber layer and the ganglion cell layer. He described large "spider and star-shaped cells." The cytoplasm stained strongly eosinophilic. In places the cell walls were indistinct, suggesting a syncytium. Many cells contained vacuoles. The nuclei were round to three cornered. There were bands and whorls of fibers which he considered "neuroglia-like."

The third such study was by Kuchenmeister (Report of Proceedings of 10th Day of Bavarian Ophthalmological Congress, München, *Ztschr. f. Augenh.*, 1935, **88**, 158) in 1935. Both eyes were involved. The optic discs were not involved. Unlike the previous reports, all layers were invaded. In places there was calcification and in the chorioid some bone formation. The cells varied in size and shape. There were "new formed glial fibers."

On March 17, 1935, a white male, 20 years of age, was admitted to the Rhode Island Hospital with pneumonia. A history of epilepsy was obtained and it was later learned that he had been under treatment at the skin out-patient department for adenoma sebaceum of the face. There was failing vision of the right eye and, on ophthalmoscopic examination, a tumor 3 mm. in diameter was seen over the optic disc.

At postmortem examination typical lesions of tuberous sclerosis were found, including multiple brain tumors, rhabdomyoma of the heart, lipofibroma of the kidney and adenoma sebaceum of the Pringle type. In the right eye was a single, raised, whitish tumor, 3 mm. in diameter, which covered the upper and inner two-thirds of the disc.

Histological preparations show the tumor overlying the optic disc and, as in Kuchenmeister's case, involving all layers of the retina. It also invades a portion of the optic nerve. In the central portion of the tumor is a large, irregular mass of ossification and about this are calcium-containing concretions. The surface of the tumor is smooth; there are no buds, as were described by Van der Hoeve and Schob. At the periphery of the tumor a thin layer of tumor cells extends out over the surface of the external limiting membrane.

The cells vary greatly in size and shape. Some are almost round. Others are markedly angulated. The cell boundaries are often indistinct, suggesting a syncytium. Many cells are elongated and the cytoplasm is drawn out in a thick process. It occurs to us that this is an attempt to form rod and cone cells.

The nuclei are round or oval and vesicular. A single nucleolus is frequently prominent. Multinucleated cells are seen having from two to five nuclei.

Phosphotungstic acid hematoxylin preparations failed to show any production of glial fibrils.

The cell type is not definitely determined. Van der Hoeve suggests that these large cells are descendants of the first anlage of the retina — a kind of neurocyte, "glia-neurocyte," which has not differentiated into glia or ganglion cells. Believing that they arise from embryonic cell rests, he proposes that they be called "phakomata" from the Greek word "phakos" meaning "mother-spot." Combining these two words he arrives at the term "neurocytophakomata retinae" or "papillae."

Schob concludes that these tumors are closely related to other gliomatous tumors and that they take origin from the nerve fiber layer which normally contains glial cells. He proposes no definite name.

Feriz considers these tumors very much like those seen in the brain and believes that they are made up of atypical neuroglia-like tissue.

It seems evident that these tumors must arise from some early cell of the embryonic retina which still is potentially able to differentiate in more than one direction. It appears definite that glial fibers were present in the cases of Schob, Feriz and Kuchenmeister. None were present in Van der Hoeve's case, nor could I demonstrate any in mine. Van der Hoeve describes nerve fibers which were not seen in any other cases. In my case there is at least a suggestion of differentiation into rod and cone cells. This is not described by any other writer.

Such embryologically derived terms as "retinoblastoma," "retinal neuroepithelioma," and so on, have already been used to designate quite different tumors. In view of our incomplete knowledge at present it is perhaps wisest to speak of them only as the retinal tumors of tuberous sclerosis.

PROTECTION AGAINST TUBERCULOSIS BY EXPERIMENTALLY PRODUCED GHON TUBERCLES. Benjamin J. Clawson, Minneapolis, Minn.

Abstract. Experiments were undertaken to determine whether from the standpoint of immunity the Ghon tubercle has a good or bad effect on the further pathogenesis of pulmonary tuberculosis.

Non-progressive Ghon tubercles were produced in the lungs of rabbits by three methods: (1) by injecting a few dried clumps of BCG intravenously; (2) by injecting 2 mg. of living BCG into the lungs with a long needle through the trachea; and (3) by injecting the same amount into the lung through the thoracic wall and pleura. All such animals became allergic, as shown by a positive Mantoux test.

These rabbits with a series of control rabbits were later inoculated subcutaneously with 0.01 mg. of a virulent strain of bovine tubercle bacillus (Ravenel). Forty-five days later all animals were killed. Those having the experimentally produced Ghon tubercle showed little or no tuberculosis, while the control animals had extensive tuberculosis.

Discussion

(Dr. David Perla, New York City.) I should like to know what the regional lymph nodes showed.

(Dr. Jules Freund, New York City.) I should like to ask if titration of antibodies and tuberculin tests were made, and if so, the results. Was any one of the three methods of injecting BCG superior to the others?

(Dr. Esmond R. Long, Philadelphia.) It may be interesting to recall experiments by Dr. Bloch of the University of Chicago on the production of similar solitary nodules. He mixed tubercle bacilli with lipiodol, and the oil seemed to hold the bacilli in a single mass. He was able to show the lesions roentgenographically and subsequently watch their development. I think the lesions he produced were a good deal like those Dr. Clawson showed, although, as I recall, they were not as sharply localized on the pleural surface. It is interesting to know what the lymph nodes showed in these cases. The Ghon tubercles are the characteristic lesion of healing childhood tuberculosis, and one of the typical features of the childhood type is enlargement of the lymph nodes with caseation, more or less in contrast to what we see in the adult type.

(Dr. Theodore J. Curphey, Westbury, N. Y.) Are these experimental tubercles calcified, even after the 45 day period?

(Dr. Clawson, closing.) The lymph nodes with these localized lesions were not involved. I used living BCG on purpose in order to get a lesion which was nonprogressive. With such lesions there was no lymph node involvement. When I used another strain, a bovine strain of low virulence, the lymph nodes did become involved. Allergy was present in all. The rabbits did show a fair degree of antibody content, as indicated by agglutinins.

In reply to Dr. Freund's question as to which of the three methods is better, I would say all three methods from the standpoint of protection appeared to be about of equal value. The last method from the standpoint of producing the localized lesion was by far the best.

In reply to Dr. Curphey, the nodules did become calcified and could be seen by the X-ray. Grossly they had not gone long enough to show a calcified nodule, but microscopically calcium was present.

BRONCHIOGENIC DISTRIBUTION OF PARTICULATE MATTER: ITS SITE OF PREDILECTION AND THE MECHANISM OF TRANSFER. Herbert S. Reichle, Cleveland, Ohio.

Abstract. Observations on human lungs injected with formalin and sectioned in the coronal plane show that the site of predilection in bronchiogenic distribution of particulate matter is a transverse band area involving, on the right, the lower one-third of the upper lobe and the apex of the lower lobe; on the left, the middle one-third of the upper lobe and apex of the lower lobe. The same site is involved when rabbits and guinea pigs are injected intratracheally with small amounts of India ink. The bronchi supplying these sites on the right are the two caudal branches of the bronchus eparterialis and the first dorsal branch of the main stem bronchus hyparterialis; on the left, branches of the bronchus ventralis I (Aeby) and the first dorsal branch of the main stem bronchus hyparterialis. Approximately similar results are obtained with the animal in supine, lateral or dorsal position. Gravity, therefore, is not a sufficient cause for the production of these sites of predilection. An additional factor — of preponderant importance in the dorsal position — is the structure of the tracheobronchial tree. The dorsal aspect of the trachea is not flat; in the well preserved specimen it shows lateral grooves separated by a membrane which is thrown into high longitudinal folds. These grooves are continued into the main bronchus both by the folds and by a projecting ledge of cartilage. If the system is not flooded, this structure insures a *marginal flow* and particulate matter suspended in liquid is shunted into the lateral and dorsal branches of the main stem bronchi.

A different mechanism results when particulate matter is suspended in air. It then proceeds by *axial flow*, which is not dependent on the structure of the bronchial wall. The distribution to the lung is governed solely by the breathing space; it is therefore roughly uniform. This mechanism has been demonstrated by subjecting guinea pigs to inhalation of air charged with animal charcoal.

TYPICAL TUBERCULOUS COMPLEXES (RANKE) IN POSTPRIMARY TUBERCULOSIS OF THE LUNGS. Kornel L. Terplan, Buffalo, N. Y.

Abstract. In a paper delivered 2 years ago at the meeting of this Association in Toronto, we mentioned a few instances of certain types of tuberculous reinfection which did not support the usual conception of localization, size and spread of the so-called reinfection. Incidental findings of focal tuberculous lesions in the lungs and in lymph nodes draining the site of these foci in individuals who did not die from tuberculosis resembled very closely lesions seen heretofore only in primary tuberculosis. In the past 2 years our systematic studies on tuberculous lesions in the lungs were continued, and more cases of this type were discovered. Restricted focal lesions, which alone were examined in this series, are a more suitable material for pathogenetic analysis than extensive chronic tuberculous lesions in patients who have died from pulmonary tuberculosis.

The anatomical findings in 6 cases showing typical Ranke complexes of postprimary tuberculosis are presented. An exogenous tuberculous reinfection brought about the formation of a second Ranke complex. In all these cases it was felt on gross inspection that we were dealing with typical primary tuberculous complexes of relatively recent age. However, in addition, roentgen examination of the lungs and all the bronchomediastinal lymph nodes revealed in 3 cases entirely calcified and partly ossified complexes. In 3 other cases additional calcified and partly ossified Ghon foci were found without gross or microscopic evidence of calcified lesions in the regional lymph nodes. The histological study of all the lesions observed at gross dissection and after roentgen examination proved that in each case there were present two tuberculous complexes of decidedly different ages. In other words, the cheesy complexes seen at postmortem appeared as tuberculous complexes of a later infection, although their anatomical-histological picture did not differ from a typical tuberculous complex of primary tuberculosis. In 4 cases the tuberculous complexes were in different lungs, in 2 cases in different lobes of the same lung, but in areas with a separate lymph drainage, like the upper third of the upper lobe and the base of the lower lobe. Histological examination showed the Ghon foci and the lymph node changes of first infection in an entirely healed, calcified or ossified stage. The complexes of postprimary infection appeared in a still active tuberculous stage; all of them were caseous. In most of these, however, there was a distinct tendency to progressive encapsulation with beginning chalky changes and very slight calcification in the center. It seems probable, therefore, that these complexes of postprimary tuberculosis also tend to become gradually calcified. The older they are, the more they may resemble in their size and structure the tuberculous complexes of first infection. If, therefore, in an adult two or more calcified tuberculous complexes are found in different lobes or in different lungs, it does not necessarily mean that we are dealing with multiple primary complexes or foci. The findings reported here show rather that postprimary tuberculous complexes cannot be ruled out.

In not one of these cases was anything known of serological reactions pointing to an active tuberculous process. Further, results of tuberculin tests were not

available, as all these individuals died from different causes, such as peritonitis, mesenteric thrombosis, acute coronary disease, and so on. It is most likely that the state of immunity acquired by the first tuberculous infection had been lost entirely, so that a second tuberculous infection brought about a lesion identical with that of a primary complex.

In the literature there are only two notes that refer to somewhat similar findings; one by Schuermann, who saw a recent cheesy complex in the intestinal tract in addition to an older calcified complex in the lung, and a similar observation by Anders. In both cases, however, the anatomical picture was complicated by more active tuberculous lesions in other organs.

In the 6 cases presented here we are dealing with almost entirely closed complexes. In only 2 of these were a few hematogenous fibrous tubercles which apparently belonged to the second infection found in the spleen and liver. These all showed a distinct tendency toward healing.

Although we have examined more than 200 cases systematically in the last 4 years, and although we have encountered tuberculous complexes of postprimary infection in at least 10 instances, we feel that the material is too small to be used for estimates as to the relative frequency of this type of reinfection.

Discussion

(Dr. Herbert S. Reichle, Cleveland.) There have appeared in the past few years, and I am thinking particularly of an article recently published in the *American Review of Tuberculosis*, a number of reports of careful studies of cases in which the tuberculin tests after being positive became negative. When I use the word negative, I mean cases in which the tuberculin dilutions had been run down to 1:10 with the use of all the necessary precautions. Since we find cases in which the tuberculin test again becomes positive after the individuals are subjected to a source of possible reinfection, it would be of great interest, as Dr. Terplan has indicated, to find out whether these individuals belonged to this group in which there has been a biological healing of the Ghon complex and whether individuals are susceptible to a second Ghon complex.

(Dr. Jules Freund, New York City.) I should like to say something that is confirmatory of Dr. Terplan's paper. I have had an opportunity to test 500 nurses in the last 4 years, and also a large number of medical students from year to year. I have found a few negative reactions in people who have definite evidence of a former infection. Tests were made with 1 mg. tuberculin at yearly intervals. Sometimes these persons were positive at the first test, and a year later were negative. Some were tested for 2 or 3 years and were constantly negative. I cannot give the exact figures at present.

(Dr. Terplan, closing.) With regard to Dr. Reichle's discussion, I should like to say that, in a series of children examined, I found a number of Ghon foci which had completely healed. Although complete serial sections were made, no signs of tuberculosis were found in the regional lymph nodes which drained the site of those foci. In only 2 of these were results of the tuberculin reaction available; in these the reaction had been negative in the year previous to death. One child died from a brain abscess and the other from uremia. It is not improbable that repeated serial tests in older children and young adults would show a considerable number of negative tuberculin tests which at previous examinations had been positive.

THE LEUKOCYTIC RESPONSE IN TUBERCULOUS RABBITS FOLLOWING THE ADMINISTRATION OF TUBERCULIN. William H. Feldman and (by invitation) Joseph Stasney, Rochester, Minn.

Abstract. In an attempt to obtain experimentally a leukocytic response comparable to the so-called "leukemoid" reaction described as occasionally associated with tuberculosis of human beings, a group of ten tuberculous rabbits was studied. The hematological observations were restricted to the quantitative and qualitative characteristics of the white blood cells. The results indicate quite definitely that tuberculin given to sensitized rabbits provokes an elevation of the leukocytic count which is often of striking proportions. The increase is predominantly granulocytic in character and there occurs a marked shift to the left. The definite hyperplasia of the bone marrow, mitosis of the immature myeloid cells of the peripheral blood and other significant changes suggest a condition similar to the "leukemoid" reaction. The monocytes participate in the leukocytosis rather insignificantly, while the number of lymphocytes shows a definite tendency to diminish during the reaction that follows the administration of tuberculin. Tuberculin given to non-tuberculous rabbits has no significant effect on the white cell count.

Discussion

(Dr. David Perla, New York City.) I should like to know what happened to the normal rabbit. I did not notice whether you mentioned it. Also, have you any data on the quantity of nuclear protein in the OT you used, because it has been shown that nuclear protein stimulates granulocytic formation in the bone marrow?

(Dr. Theodore J. Curphey, Westbury, N. Y.) I should like to comment on Dr. Feldman's work in connection with some similar experiments that Dr. Russell and I performed in human beings a few years ago in attempting to study the changes in the bone marrow response in tuberculous patients following an intracutaneous injection of old tuberculin. The idea was to develop a functional hematopoietic test in this disease, similar to the glucose tolerance test in diabetes. We thought we could show a correlation between the extent of the shift to the left and the degree of involvement of the lung. For a while we thought the response was a specific one, but we were surprised to see that similar changes could be produced by the use of sterile milk in patients with the production of curves quite identical to those obtained by the use of OT. This, I think, will partly answer Dr. Perla's question.

(Dr. Kornel Terplan, Buffalo.) My interest in Dr. Feldman's experiments concerns primarily their application to human pathology. In certain types of chronic miliary tuberculosis bone marrow reactions of a leukemic or leukemoid nature have been observed. I remember one particular case the blood films of which were seen by the best authorities in hematology and diagnosed as leukemia. When the patient died, however, he showed only chronic hematogenous tuberculosis from which he had suffered for a half year. There were extensive lesions in the lungs, liver and spleen. The bone marrow did not show tubercles, and there were no leukemic changes in the organs mentioned.

(Dr. F. E. Chidester, Boston.) May I suggest that the use of milk may initiate the same type of reaction obtained with tuberculin and cause the very same effects on the glands of internal secretion, particularly those eliminating iodine, and that the relation of the thyroid to the problem of immunity has been ignored successfully, but not skillfully, since the days of Sajous?

(Dr. Feldman, closing.) In answer to Dr. Perla's question about the normal or the non-sensitized rabbits, these failed to give any change after the administration of OT. I cannot tell you as to the quantity of tuberculo-protein. We used tuberculin prepared by Parke & Davis & Co., and Dr. Long kindly sent us some PPD which we used on a small series. We did not have enough of his material for more, because it takes quite a lot of material with the doses used in this study. We do not feel this is an example of a specific reaction. We think other agents may elicit this sort of response, and we are at the present time investigating that angle of it.

I refuse to become engaged with the subject of Dr. Chidester's remarks — I do not know anything about it.

THE SIMILARITY BETWEEN THE REACTION TO SILICA AND TO TUBERCLE BACILLI.
Leroy U. Gardner, Saranac Lake, N. Y.

Abstract. The primary reaction to both silica and tubercle bacilli is phagocytosis by mononuclear leukocytes of the histiocytic type. The morphological, tinctorial and supravital staining reactions of the cells responding to these irritants are identical. In both silicosis and tuberculosis the histiocyte is modified to form epithelioid cells and giant cells of the Langhans type. Both irritants cause necrosis of tissue with the liberation of free fat and an exudation of leukocytes and serum. In each case the necrotic matter may undergo subsequent calcification. Both stimulate proliferation of histiocytes and fibroblasts that are laid down in the form of a compact nodule. About the periphery of each lesion is a more or less well defined zone of lymphocytes.

Differences between tubercle and the silicotic nodule are due to the nature of the irritants. Tubercle bacilli are alive and capable of indefinite multiplication but their rate of growth is more or less constant and is quite well balanced against that of the host's cells. As a consequence all *primary* tubercles are very similar in structure. As soon as excessive numbers of bacilli have formed, the equilibrium is disturbed, the tissues degenerate and the organisms spread into new areas. The character of the silicotic nodule varies with the number and size of the silica particles that have been concentrated in a focal area. Large numbers of very fine particles produce nodules with extensive central necrosis; smaller quantities of somewhat larger particles cause proliferation with little or no degeneration. Tubercles spread by direct extension and by metastasis through blood and lymph vessels and duct systems; silicotic nodules are enlarged gradually by peripheral expansion. Metastasis is generally limited to the regional lymph nodes. The fibrosis of silicosis is unique and is characterized by a peculiar hyaline swelling of the intercellular collagenous fibers. This is possibly a protective reaction that prevents diffusion of toxic material from the interior of the nodule.

The fact that a simple inorganic compound like SiO_2 can set in motion a complicated series of cellular reactions comparable to those produced by a living organism made up of proteins, lipoids and carbohydrates is cause for wonder. It challenges the generally accepted views on the action of the tubercle bacillus. It suggests a search for a common physiochemical change produced in the tissues by the lipoids of the bacillus and by the molecule of silica. Both of these irritants are relatively insoluble but both theoretically excrete small amounts of toxic material over prolonged periods. Since the exact nature of the injury produced by each of these substances is still unknown it is profitable to correlate any relevant data.

Discussion

(Dr. Thomas H. Belt, Toronto.) Dr. Gardner's interesting work is of especial interest to those who have been working with silicosis, and I think it touches upon a problem which is of vital importance in connection with pneumoconiosis, namely the distinction between tuberculous lesions and the so-called "dust" lesions. As those pathologists who have examined the lungs from cases of silicosis well know, the majority of these people die with both active and healed tuberculosis in their lungs, and it is often an extremely difficult problem to distinguish which lesions are due to the silica or to the dust, and which are due to the tubercle infection. The chief difficulty arises in distinguishing the healed tuberculous nodule from the silicotic nodule, and in many cases I think it is impossible to make a satisfactory distinction between them. Dust particles will often be found incarcerated within the confines of a healed tuberculous lesion. One can find dust in appreciable quantities in both the healed and active tuberculous lesion; and in those cases of pneumoconiosis where death is due to extensive tuberculosis superimplanted, one can see all stages of the tuberculous lesion, right from the earliest caseous or proliferative cellular lesion to the healed nodule. It is difficult indeed, as I have stated, to say definitely which are silicotic and which tuberculous nodules. I have often been led to wonder with other people, notably Kettle, and some of the other pathologists who have worked on silicosis, whether or not the majority of the silicotic nodules may not be really healed tubercles modified somewhat by the presence of the dust; but, one suspects in many cases it represents a lesion due to both the tubercle bacillus and the dust. I should like to ask Dr. Gardner about these lesions which he has produced in rabbits, the beautiful illustrations of which show giant cells and a very cellular reaction. Is there not some possibility that there may have been an element of infection in them, not necessarily tubercle infection? I should also like to know whether or not he has stained these lesions for bacteria and if he thinks they are due entirely to dust particles.

(Dr. Herbert S. Reichle, Cleveland.) In Cleveland we have exactly the same difficulty in distinguishing between pneumoconiosis and tuberculosis of the chronic fibroid type which Dr. Gardner has demonstrated so beautifully in his animals. I believe there are a fair number of cases in which, so far as one can ascertain, there is no tuberculosis. Certainly, so far as examination of the cavities can be made, they show nothing that is typical of an active exudative tuberculosis. Some of these patients have been examined for months in a sanatorium and their sputum, which is quite plentiful, never contained tubercle bacilli. In some cases this sputum has been put into animals and they have not succumbed to tuberculosis. I think it is therefore quite reasonable to suppose that pneumoconiosis can of itself give rise to serious conditions. I am thinking of one particular individual in whom there was a cavity of such a size as to erode the pulmonary artery with a subsequent fatal hemorrhage.

There are two points which seem of some importance in differentiation. The first is the absence of perifocal inflammation, and the second the unusual clarity with which the band area is found in pneumoconiosis. I think if the lungs are examined by coronal section you will find in pneumoconiosis the greatest amount of disease in the band area, relatively little at the base, and very little at the apex.

(Dr. Esmond R. Long, Philadelphia.) I am interested in the amounts of silica you see in the monocytes and in the epithelioid cells that develop from them.

Are they comparable? Have you any evidence as to whether silicate has been formed from the silica in the epithelioid cells? Possibly evidence may be obtained from such a technic as microincineration. The epithelioid cell, the characteristic cell of tuberculosis, represents the response of the body to substances with special physicochemical properties, like the lipoids of tubercle bacilli. The cytoplasm is essentially an emulsion of protein, lipoid and water, and here it appears to be possible that we have an emulsion of protein, water and silica.

(Dr. Ellis Kellert, Schenectady, N. Y.) In view of the medico-legal implications of what Dr. Gardner has told us, may I ask if he feels that the presence of silica in the lungs stimulates the progress of tuberculosis, and what effect does it have on the picture of tuberculosis in the lung?

(Dr. Gardner, closing.) Dr. Belt has commented on the difficulty of distinguishing tuberculous lesions from those produced by silicosis. The typical reactions to tubercle bacilli and to silica present no particular problem in diagnosis. Small sections of isolated lesions may be more troublesome. It has been my opinion that probably a great many of the healed tubercles one sees in lungs and peribronchial lymph nodes are influenced by silica particles which tend to gravitate toward these lesions and are retained in their outer walls. This dust modifies the fibrous tissue of the tubercle. We do not generally see healed tubercles in other organs, such as the spleen, with the same kind of hyaline fibrosis arranged in definite laminas that are encountered in lung tissue. Since it is common knowledge that carbon tends to be deposited about immobile structures like tuberculous scars and since it is well known that the amount of silica in the ash of living tissue increases with advancing age, it seems quite probable that silica may accumulate in the periphery of tubercles and produce the hyalinized fibrosis.

As to the question of the necessity of an element of infection in the silicotic lesion, I may say that I am now firmly convinced that silica alone can produce nodular fibrosis. When we first began to discover such reactions in guinea pigs inhaling silica over long periods of time we were amazed at the close resemblance the foci bore to tuberculosis and thought that possibly we were dealing with accidental superimposed tuberculous infection. We therefore skin-tested 200 guinea pigs but never found a positive reaction.

To exclude other infections, we have made Gram stains in an attempt to discover any organisms that might be present in the lesions, and have subinoculated and cultured them, but have never found bacterial infection entering the picture except in cases of obvious pneumonia. One can produce necrosis and fibrosis by injecting sterile suspensions of silica particles into the subcutaneous tissues, and after intravenous injection of pure silica animals develop typical silicotic nodulation in the bone marrow, liver and spleen.

Dr. Reichle brought up the question of cavities in the silicotic lung. In my experience one is apt to see cavities in the so-called conglomerate massive fibrosis of silicosis which are produced as a result of deposition of inhaled silica in portions of the lung previously injured by infection. Perhaps they are unresolved pneumonias similar to those which Dr. Haythorn described in the anthrasilicosis from his locality. Occasionally the cases of this type show central cavity-like areas of degeneration, but the antra do not have definite walls or trabeculae and they do not look like tuberculous cavities. On the other hand, there are cases of perfectly typical tuberculosis associated with silicosis with cavities in which one cannot demonstrate the tubercle bacilli. We have inoculated the cavity contents in a good many cases that showed typical tuberculous histology and I can

recall about 5 in which the guinea pig failed to develop tuberculosis. We have had the same experience in guinea pigs exposed to silica and infected with tubercle bacilli. The infection would progress and would eventually kill the animals, but sometimes subinoculation of their tissues failed to demonstrate living organisms. I had thought for a time the organisms had lost their acid-fastness in the presence of silica but now I am not sure this is true.

Dr. Reichle spoke about a band across the lungs where silicotic and other lesions tend to localize. I would disagree with him, for I believe that silica or any foreign particles borne in the inhaled air are distributed uniformly throughout the lung. We fail to see evidence of the reaction to silica as nodules both in the bases and sometimes in the apices of the lung because of the emphysema which develops subsequently and displaces the normal relations. The characteristic picture of the silicotic lung shows clear costophrenic regions due to the emphysema which comes on after the nodules have developed in the rest of the lung.

Dr. Long asked about silicate formation inside the monocyte. I do not know whether silica dissolves and recombines with bases in their cytoplasm. Dr. Irwin, who is sitting beside me, will possibly care to discuss this question, as it is in line with some of the work that he has done.

Dr. Kellert asked about the stimulation of tuberculous infection by the presence of silica. We have plenty of experimental evidence and a great deal of clinical evidence to indicate that silica dust does reactivate latent tuberculous foci and cause them to become progressive. Probably 60 to 75 per cent of persons with silicosis ultimately die of tuberculosis. What proportion are infected from exogenous sources after the development of the silicotic nodulation is not known; probably many of them are due to reactivation of preexisting latent foci. Other types of dust do not have the same stimulating effect on tuberculous infection.

TUBERCULOSIS OF THE PROSTATE. Robert A. Moore, New York City.

Abstract. The prostate in 163 cases of tuberculosis has been studied by the step-section method. A total of 20 instances of tuberculosis of the prostate was found. Correlation with the presence of tuberculosis in other urogenital organs gives no support to the theories of ascending or descending tuberculosis of either the genital or urinary tract. There is strong pathological evidence that tuberculosis of each of these organs is hematogenous except in a rare instance.

PATHOLOGICAL FEATURES FOLLOWING SHOCK WITH DELAYED DEATH. Virgil H. Moon and (by invitation) David R. Morgan, Philadelphia, Pa.

Abstract. Physiological features compiled from experimental studies on shock may be combined into a useful definition. Shock is a circulatory deficiency, neither cardiac nor vasomotor in origin, characterized by decreased volume of blood and cardiac output and increased concentration of the blood. Regularly associated with it are low blood pressure and low basal metabolism. The coagulability of the blood, the blood chlorides and the renal excretions are diminished. The cardiac rate and the non-protein nitrogen of the blood are increased. Variations in respiratory rate, temperature and some other phenomena are frequent, but not consistent.

This syndrome is accompanied by characteristic visible pathological changes. There is diffuse congestion of the viscera, especially of the lungs, the mucous and serous surfaces, the liver and kidneys. Frequently the serous cavities contain blood tinged fluid. Ecchymoses are often numerous and widespread. Microscopic examination shows marked engorgement of the venules and capillaries and

minute extravasations in each of the tissues mentioned. The spleen is usually contracted and relatively anemic. These changes are regularly present when death occurs early. Edema develops when death from shock is delayed somewhat. This is especially marked in the lungs and mucosae.

We have found these changes following death by shock in a wide variety of clinical conditions, including surgical shock, burns, eclampsia, toxic jaundice, intestinal obstruction, poisoning with barbiturate compounds, and following severe infections.

Several of these conditions were reproduced experimentally by implanting muscular substance into the peritoneal cavity, by cutaneous burns, by intestinal obstruction, by injections of bile and bile salts, by barbital poisoning, and by injections of histamine. Circulatory failure and hemoconcentration preceded death in each instance. Pulmonary edema was a prominent feature in these experiments. The edema fluid had a specific gravity approximating that of the blood serum. When death was delayed several days, secondary pneumonia developed regularly in the edematous lungs. This had the same gross and microscopic features that we have reported (The Origin of One Type of Secondary Pneumonia, *Am. J. Path.*, 1933, **9**, 899) occurring under similar conditions in man.

Discussion

(Dr. Walter Cannon, Boston.) I have been much interested in the results which Dr. Moon has shown. During the World War we came upon the phenomenon of hemoconcentration, and found in some cases of shock the blood count went as high as 7,000,000 or more per cmm. I was interested also because of the evidence he offered as to a mystery that has confronted persons who have worked on the problem of shock for many years. It has been clear for a long time that the central phenomenon in shock is a diminution of blood volume, usually in severe cases to such a degree that less than the minimal capacity of the circulatory system is present. With this diminished blood volume the blood pressure necessarily falls. The query has always arisen, where is the lost blood? That is sometimes spoken of as "the mystery of the lost blood." The concentration of the corpuscles found in the skin and also in the capillaries of the intestinal villi indicates that there must be a corresponding plasma somewhere. Dr. Moon's evidence as to its extravasation in the places he described this afternoon is a solution, or a partial solution, of this problem.

(Dr. David Perla, New York City.) I have had considerable experience in shock produced by histamine injections in rats over a number of years, and I can fully confirm Dr. Moon's observations, although I have not systematically studied that phase of the problem. I always noted that the animals which died following the injection of histamine, particularly if they died later than a few hours after injection, showed effusions in the pericardial sac and in the peritoneal and pleural cavities. Under these circumstances the blood always had a definite inspissated appearance: it was markedly viscid. I think Dr. Moon's suggestion that there must be a universal change in the permeability of the capillaries and a relative concentration of the red blood cells in the circulating blood is true.

(Dr. Harry Goldblatt, Cleveland.) I should like to ask Dr. Moon just what criteria he has used for the determination of the existence of hemoconcentration and would be pleased if he would define this word as he uses it. The mere presence of an increased number of red blood cells and a corresponding increase in the hemoglobin are not proof of hemoconcentration in the true sense of the word.

Concentration of the blood due to loss of water would be accompanied by changes in the concentration of serum protein and of other substances contained in the plasma. The injection of peptone intraperitoneally produces an effect identical with that which follows the injection of chopped muscle mentioned by Dr. Moon. There is a great increase in the number of the red blood cells and a corresponding increase in the amount of hemoglobin, but there is no other evidence of hemoconcentration. The effect may result from the sudden entrance of red blood cells from a storehouse, such as the spleen, and need not be due to loss of water from the blood.

(Dr. Paul Klemperer, New York City.) I should like to ask Dr. Moon if he examined the liver in his experiments. In his last paper he mentions edema of the liver in only one of his dogs. I am very much interested in this question because in my presentation later to-day I will deal with a related topic. We had only 3 cases of surgical shock in which we examined the liver particularly and did not find edema.

(Dr. Paul R. Cannon, Chicago.) Dr. Holck and I have observed in rats injected with nostal and pernocton that the "delayed death" which occurs at about the 5th day after injection may be explained by certain fatty degenerative changes in the lungs, myocardium, liver and kidneys. Most of the animals developed pulmonary edema and pneumonia, and I was particularly interested in the fatty degeneration of the walls of blood vessels in the lungs. There was obviously a profound toxic injury to the vascular walls, and this injury may be similar to that described by Dr. Moon in explaining "medical shock" as due to capillary atony and increased capillary permeability with hemoconcentration.

(Dr. Walter Cannon, Boston.) So far as the term hemoconcentration is concerned, I am not defending it. I took it over from Dr. Moon temporarily. What we reported in 1918 was a concentration of the corpuscles; we did not go beyond that. We made comparisons of blood taken from peripheral areas in various parts of the body and blood taken simultaneously from the veins of persons in shock. There was a marked discrepancy between the concentration of the corpuscles in the two regions. The corpuscles in the skin areas were very much more concentrated than those taken from the veins. We made no observations on whether there was a loss of water from the blood and therefore a concentration of blood proteins.

(Dr. Moon, closing.) I am deeply gratified by the discussion and the interest shown and am indebted particularly to Dr. Cannon for his interesting remarks. Many of the phenomena we have reported were those observed by him and his colleagues during the World War.

As to the presence of effusions, they are regularly present in acute shock. If the shock is somewhat delayed, we find fewer effusions in the serous cavities and more edema in the lungs and mucosae, but in acute shock, fatal in 24 hours, it is common to find a very marked effusion in the serous cavities. The fluid is blood tinged in the majority of instances.

Regarding the problem of the lost blood, I recall one instance in which shock terminated fatally in 36 hours. The lungs weighed 1200 gm. above normal, due to congestion and edema, and there were over 1500 cc. of bloody fluid in the serous cavities, to say nothing of the edema of the mucosae and other tissues. Those two locations accounted for the loss of almost 3 liters of fluid from the circulation. The lost blood in shock may be accounted for by the effusion, edema and visceral congestion.

Regarding the criteria for hemoconcentration, our interpretation is that which

Dr. Cannon has just made, an increase in the number of corpuscles per cmm. of blood. It may be determined by the blood count or hematocrit.

I am glad Dr. Goldblatt mentioned the effect of peptone, because we did not have time to mention all the agents which will produce shock. A list of the agents causing shock would include peptone and tissue extracts, as well as various poisons. The presence of a higher concentration of the blood in peptone poisoning provides another instance of what we have already stated.

In reply to Dr. Klemperer, the liver in shock is characteristically congested and sometimes edematous. The liver will drip blood freely when sectioned and pathologists have interpreted it, perhaps incorrectly, as passive congestion of the liver. The same statements apply to the kidneys.

I have not looked for fatty degeneration of vessel walls. We were concerned with the edematous, hemorrhagic and congestive features and have not gone into the more minute details of changes in the endothelial cells in shock.

THYROTROPIC EFFECT OF CRETIN RATS' PITUITARIES CONSIDERED IN RELATION TO PITUITARY HISTOLOGY. Isolde T. Zeckwer, Philadelphia, Pa.

Abstract. The pituitaries of cretin rats were injected into young female guinea pigs on 3 or 4 successive days, and the results compared with the effect produced by similar injections of pituitaries from normal rats of the same age and sex. The histological hyperplasia of the guinea pig thyroids was about the same after pituitary cretin injections as after normal pituitary injections. The absolute weights of thyroids were increased after cretin pituitary injections, but were slightly less than after normal pituitary injections. If calculations are made for the body weights of the rats, the dwarfed cretin has available more hormone for each gram of its body weight than the normal rat. If calculations are also made for the weights of the injected pituitaries, each milligram of the heavier pituitary of the cretin contains about the same amount of hormone, per gram of body weight, as each milligram of the normal.

The cretin pituitary contains large numbers of cells with fine blue granulations and intracellular hyaline material which are histologically very different from "castration cells" which contain coarser, darker granules, with intracellular hyalin of a different character. In pituitaries of rats both thyroidectomized and castrated at the same time, the two types of cells can be differentiated. The cells that react to thyroidectomy may be the producers of the thyrotropic hormone. The intracellular hyaline material, however, seems in excess of any increase in amount of thyrotropic hormone calculated by assay.

Discussion

(Dr. David Seecof, Montreal.) Perhaps this point was presented, and I may have missed it. I should like to inquire about the time factors in relation to the hyaline droplet cells. How soon after the experiment did they appear, and how long after did they remain?

(Dr. H. E. MacMahon, Boston.) In a recent paper with the title, "A Change in the Basophil Cells of the Pituitary Gland Common to Conditions which Exhibit the Syndrome Attributed to Basophil Adenoma," Dr. Crooke of England demonstrated hyaline changes in the basophil cells of the pituitary gland which he considered to be an expression of altered physiological activity. It was the only conspicuous abnormality common to all twelve examples of basophilism

and he regarded it as the one abnormality of fundamental significance. I should be very much interested in knowing whether Dr. Zeckwer has had an opportunity of comparing the hyaline changes which she has described with those recently reported by Crooke.

(Dr. Zeckwer, closing.) The hyaline material appears in a few weeks time. It is well developed in a month. The last slide of the pituitary was from a 44 day rat, and you see about half of the pituitary is filled with hyaline material. We have studied them up to a year, and up to a year the hyaline material remains. The most pronounced changes are earlier, from a month to 2 to 3 months.

In reply to the second question, I saw Dr. Crooke's sections of pituitary when he came to Philadelphia on a visit. I did not see any of his sections of Cushing's syndrome, but I saw those from cases of Addison's disease in which there were basophilic cells with very small vacuoles in them, not at all like the cells in these cretin pituitaries. The basophilic cells were very different, having a sort of foamy, fatty vacuolated appearance. I have not seen sections of Cushing's syndrome.

THE RELATION OF THE HYPOPHYSIS TO THE SPLEEN. David Perla, New York City.

Abstract. Removal of the hypophysis in adult rats is followed by progressive atrophy of the spleen. At the end of 2 months the ratio of spleen weight to body weight is one-half the normal. The administration of hypophyseal emulsion repairs to a considerable degree the atrophy of the spleen in such animals.

Hypophysectomy completely inhibits the regeneration of splenic tissue after partial splenectomy. Administration of anterior hypophyseal emulsion restores to normal the regenerative capacity of splenic tissue of the hypophysectomized rat.

The daily administration of anterior hypophyseal emulsion of cattle or of alkaline extracts of fresh or acetone-dried anterior hypophysis during a period of 10 days in normal *Bartonella*-carrier or *Bartonella*-free rats results in hypertrophy of the spleen to twice the normal size. Normal rats receiving emulsion during a period of 1 month become refractory to the spleen-stimulating effect. The spleen shows little increase in size above the normal at the end of this period. Injections of horse serum or of alkaline extracts of acetone-dried kidney, and spleen or liver of cattle do not cause enlargement of the spleen of rats from *Bartonella*-free stock.

The increase in the size of the spleen, following daily administration of emulsion of the anterior hypophysis, is due primarily to a marked hyperplasia of the reticular and endothelial cells of the red pulp. The follicles also increase in size. Clusters of reticular cells containing numerous lymphoid elements appear throughout the splenic pulp. The reticular tissue of the bone marrow is similarly increased. There is a striking increase in the number of hemocytoblasts and megakaryocytes. The Kupffer cells are not affected.

The spleen-stimulating factor is not present in the acid extract of anterior hypophysis that contains thyrotropic and adrenotropic factors. It is present in some degree in an alkaline extract of fresh anterior hypophysis containing growth and gonadotropic factors. It is also present in alkaline extracts of acetone-dried anterior hypophysis relatively free from growth hormone.

The presence of a spleen-stimulating factor in the anterior hypophysis is suggested by these experiments.

Discussion

(Dr. Thomas H. Belt, Toronto.) Did you make observations on other organs beside the spleen in these experiments? What calls that to mind is the syndrome known as Simmond's disease in the adult human, where there is a destruction of the anterior lobe of the pituitary and in which other organs are nearly always reduced considerably in size, and conditions in which there is an over-production of the anterior lobe hormones, as in acromegaly, where nearly all the organs are increased in size.

(Dr. Perla, closing.) I do not understand if you meant in the hypophysectomized animals. In them, of course, changes in other organs than the spleen are observed. Many have been reported by numerous investigators, particularly by Smith, who has demonstrated atrophy of all the endocrine glands following removal of the hypophysis, with a corresponding shrinkage in the organs in proportion to body weight. Conversely, the hepatomegaly associated with acromegaly is very variable. In our animals that received alkaline anterior hypophyseal extracts (I did not have time to stress the effect on the other tissues) there did not seem to be produced a corresponding change in the kidney, thyroid or adrenal glands. There was some increase in the gonads of the animals when such alkaline extracts were given.

THE IDENTIFICATION OF TUMOR CELLS IN SEDIMENTS OF SEROUS EFFUSIONS. Nathan Chandler Foot, New York City.

Abstract. Two methods are commonly employed for identifying tumor cells in serous effusions: (1) The sediment is smeared out and stained in the dry, fixed, or moist state in the usual way, or supravitally in fresh smears. This does not concern us here. (2) Fluid is sedimented by gravity and the concentrate is then centrifugated and the resulting button fixed, embedded in paraffin and sectioned like tissue. This is the method first published by Mandelbaum in 1900, and discovered and revived by many of us, as Graham has detailed in his article in the *American Journal of Pathology*, 1933, **9**, 701.

Zemansky reviewed the results obtained by this method at the Mount Sinai Hospital in New York and formulated criteria for making positive diagnoses of tumor cells, using their experience and drawing on the meager literature. According to him, tumor may be inferred from the presence of: (1) fragments of tissue with a definite arrangement of cells and stroma; (2) multiple clumps of large, deeply staining cells which give the slide a mottled appearance; and (3) finer cellular changes, such as extreme irregularity in size and shape, eccentricity of the nucleus, extremely large nuclei and nucleoli, multinucleation and typical or atypical mitotic figures.

Dissatisfied with the results we were obtaining at the New York Hospital, I undertook to review our series of 55 specimens of ascitic and 85 of pleural effusions. Pericardial fluids had been examined but were uniformly negative. The analysis consisted of tabulating the results of the original examinations as based on clinical history, morphology, and the criteria just stated. This tabulation was followed by a check-up on morphological grounds alone, using the same criteria, but neglecting the clinical history and ignoring the name of the patient, which might be familiar and afford a clue. Special stress was laid on the reliability or unreliability of the morphological criteria. It was found that mitoses occurred in 26 per cent of the slides examined and tumor fragments in 15 per cent. Typical mitoses were, then, found in definitely tumor-negative sections, although

monster or atypical mitoses were never found except in positive cases. Since multinucleation was observed in 65 per cent, irregularity of outline and staining in 43 per cent, and clumping of cells in 54 per cent, these criteria are not of much value.

It was found that desquamated mesothelial cells formed the chief source of error, being easily mistaken for tumor cells. The N/n ratio, lately stressed by MacCarty in this country and employed somewhat differently by Quensel and Karp abroad in connection with smeared sediments, was worked out in 44 sections checked by biopsy or autopsy. Using Quensel's simple method of dividing the longest nucleolar diameter by the longest nuclear, figures were obtained that completely confirm those he and Karp had found. An n/N ratio below 0.20 indicates no tumor present; one of 0.25 plus is indicative of a tumor-positive diagnosis; there is a "no-man's land" between 0.20 and 0.25, where the other criteria are also unreliable and the best one can do is to sum up the lot and draw tentative conclusions. Given the other way around, N/n, by dividing the nuclear diameter by the nucleolar, 0.20 becomes 5:1; 0.25 becomes 4:1, and so on. The ratio of the squares of these figures (roughly approximating their area) is obvious; the cubes, approximating volume, are 125:1; 64:1; and, in the case of our highest figure of 0.40, 15.6:1. Various methods are used for working out the areal or volumetric ratios. As the mere use of the longest diameters gives correct diagnoses in about 70 per cent of the cases, without any other data whatever, it may be inferred that this would suffice. Karp used the formula for calculating the area of an ellipse, taking the longest and shortest diameters of nucleus and nucleolus. His figures do not differ materially from Quensel's.

Summing up, we may say that the following criteria have been found useful: the presence of tumor fragments with stroma, monster mitoses, extreme irregularity in size and shape of the cells in question, the nucleo-nucleolar ratio and a liberal background of clinical data and judgment of morphology obtained through experience.

Normal mitoses, cell clumping, eccentricity of nucleus or multinucleation have been found to be unreliable criteria.

An n/N ratio of 0.20 or under (Quensel), or 1:125 by the volumetric method (MacCarty), indicates the absence of tumor cells; one of 0.25 or over or 1:64, by the volumetric method, is almost pathognomonic of the presence of tumor.

Lymphoid tumors are diagnosed best by morphological means, as are the myeloid; the n/N ratio is of chief value in the case of carcinoma.

Discussion

(Dr. Shields Warren, Boston.) I wish to express my thanks to Dr. Foot for this clear exposition, because we are frequently called upon to try to settle this very puzzling problem. I have myself relied somewhat on the concomitance of red blood cells and wonder if he found them of importance in the course of his investigation.

(Dr. David Seecof, Montreal.) In 1924, in a publication of the New York Pathological Society, by relying on the criteria of malignancy such as we all use, I showed that about 70 per cent of the diagnoses were correct. Without using any special criteria, the figure arrived at was practically the same as Dr. Foot just gave.

(Dr. Howard T. Karsner, Cleveland.) I wish to ask Dr. Foot one question, and it must not be interpreted as showing any lack of appreciation of the

thoughtful care and precision used in this work. The question is this: If the findings of the nuclear-nucleolar ratio are in favor of tumor and all the other findings fail to support that conclusion, what diagnosis can be made?

(Dr. Foot, closing.) In reply to Dr. Warren's question as to the red blood cells, we usually add enough glacial acetic acid to our fluid to make it up to 2 to 3 per cent. I have been trying to check up on the point he brings up and found a surprising number of red blood cells in negative fluids, so that I do not think they are of very great importance.

In regard to Dr. Seecof's remarks, Zemansky's percentage was very much better in positive cases of tumor and very much worse in cases that he diagnosed as negative and which were proved to be positive. We have at least improved the negative side of the picture, and I think the whole thing depends on the recognition of changes that take place in the mesothelial cells, which apparently grow rather luxuriously in ascitic and pleural fluid, such fluid acting as a sort of nutrient medium for them, as we often find mitotic figures and other definite qualities of tumor cells.

As to Dr. Karsner's question, that situation sometimes arises, and at the present moment constitutes something of a quandary. I think that if we had a careful negative clinical history and all our morphological data favored a negative report, and if the nuclear-nucleolar ratio favored a positive report, we should certainly scrap the ratio in that case. I am not trying to advocate this ratio as a single method. We must apply all the methods we have. Even then we will find it hard to make the diagnosis in a great many of the cases which lie in the middle ground and contain a great many desquamated cells.

Osseous Metastases of Carcinoma of the Prostate, with Special Reference to the Perineural Lymphatics. Shields Warren and (by invitation) Paul N. Harris and Roger C. Graves, Boston, Mass.

Abstract. Skeletal metastasis of prostatic carcinoma has been generally attributed to blood borne emboli, but the disproportionately frequent involvement of the pelvis and lumbar vertebrae cannot be satisfactorily explained on this basis. In 7 cases of carcinoma of the prostate and 1 case of carcinoma of the bladder, all the pelvic viscera and soft tissues, with sacrum, bodies of lumbar vertebrae, and adjacent parts of pelvic bones, were removed in a block. After fixation in formalin, horizontal cuts were made at 5 mm. intervals and large sections made. These cases were supplemented by sections of a number of surgical or other autopsy specimens of prostatic carcinoma.

Perineural invasion was not found in the bladder carcinoma, but was present in all the prostatic tumors. In 6 of the 7 prostatic cases perineural invasion was extensive and could be traced up the sacrum and vertebrae. In the other case spread of the tumor seemed to be by lymphatic embolism. Perineural and lymphatic plexus invasion was seen in the periosteum, and direct invasion of the cortical ostia by tumor extending in from these perineural masses was seen.

Perineural invasion is not peculiar to prostatic carcinoma, but is conspicuous because of the abundance of nerves in and about the prostatic capsule.

The occurrence of pain in prostatic carcinoma and its bone metastases may be due to perineural invasion.

The importance of lymphatic and hematogenous metastasis is admitted, but it is thought that the perineural lymphatics offer the most probable means of invasion of bone and that the disproportionately high incidence of metastasis to pelvis and lumbar vertebrae is probably due to invasion by this channel.

OSTEOID OSTEOMA. Henry L. Jaffe, New York City.

Abstract. In the last 3 years I have seen 11 instances of the bone lesion about to be demonstrated. The principal clinical and radiographic features of this lesion can be summarized as follows:

(*a*) The patients were all adolescents or young adults.

(*b*) The principal complaint was local pain.

(*c*) Uniformly, the lesion originated in spongy bone areas.

(*d*) As observed radiographically, the pathological areas were roundish and clearly circumscribed.

(*e*) The lesions were small and closely similar in size.

(*f*) In every case operation was performed on the assumption that the lesion was an inflammatory one.

(*g*) Complete eradication resulted in the eventual disappearance of all symptoms, without recurrence of the local condition.

These features reappear from case to case with such regularity that they clearly seem to constitute a clinical syndrome. This fact, in conjunction with the presence of certain special pathological-anatomical manifestations, has led me to regard the lesion as a distinctive one and to designate it as osteoid-osteoma.

On the basis of microscopic examination of the stages of development of the lesion, it appears that the latter arises from osteoblasts. Irregularly between the osteoblasts, intercellular material develops. In this way, patches of osteoid tissue are formed. In the further progress of the lesion, the osteoid tissue becomes calcified and even converted into atypical bone. In the course of the conversion osteoblasts appear. Sometimes the lesions are rather vascular.

In presenting the material I shall begin with the cases in which the lesion is characteristic, clear-cut, and fully developed. It will then be easier to follow the demonstration of the cases that are more obscure, apparently because they are in an earlier stage of their pathological evolution.

As to differential diagnosis, osteoid-osteoma has no features suggesting (*a*) an inflammatory origin; (*b*) origin from an embryonic rest; or (*c*) that it represents an unfamiliar aspect or healing stage of a giant cell tumor, localized ostitis fibrosa, or cyst.

In closing, I wish to suggest that osteoid-osteoma is possibly the benign counterpart of the malignant osteogenic sarcoma. This is indicated by the behavior of a metacarpal bone tumor which I observed some years ago. This tumor was apparently originally an osteoid-osteoma which, because of incomplete removal, continued to proliferate and began to take on the characteristics of an osteogenic sarcoma.

The details of certain of these cases may be found in the *Archives of Surgery*, 1935, **31**, 709.

Discussion

(Dr. Kornel Terplan, Buffalo.) Are these tumors identical with the so-called osteoid-chondroma of Virchow? I had an opportunity to examine a tumor removed from the upper end of the femur in a young girl. This tumor was about the size of a small peach. The histological structure resembled closely that seen in the slides exhibited by Dr. Jaffe. As far as I know the surgical removal of this tumor resulted in complete cure; there was no recurrence of the osteoid tumor during the 4 years which have elapsed since.

(Dr. Jaffe, closing.) I read the paper of Virchow which was referred to. He

described a large tumor in the humerus, which he designated osteoid chondroma. He stated that while this tumor offered a better prognosis than osteosarcoma it might become malignant and change to osteosarcoma. In any event, I considered this lesion, which had none of the features peculiar to the condition discussed, as osteoid-osteoma.

HEPATIC EDEMA AND "SEROUS HEPATITIS." Paul Klemperer and (by invitation) Harold W. Keschner, New York City.

Abstract. In recent writings Roessle and Eppinger attribute great significance to the exudation of serous fluid into the Disse's spaces of the liver and consider edema of the liver as it occurs in infectious or toxic diseases as the chief manifestation of an acellular "serous hepatitis." They believe the liver parenchyma can be severely impaired in such conditions, either because of compression of the liver cells or because of the simultaneous penetration of hepatocellular toxins Moreover, they regard the extravasation of plasma as a stimulus for connective tissue proliferation and consider it as the initial phase of severe chronic liver alterations, especially atrophic liver cirrhosis. The conclusions of such prominent investigators were deemed worthy of investigation.

Liver sections of 505 consecutive autopsies were studied and, of these, 79 or 15 per cent satisfied the criteria of "serous hepatitis." The greatest incidence, 42 cases, was found in cardiac and nephritic states which were associated with severe hydremia. A high incidence of positive findings in malignant nephrosclerosis, uremia (other than nephritis), diabetic coma, influenza and Graves' disease was noteworthy.

Contrary to Roessle, a surprisingly low incidence occurred in such infectious or toxic conditions as sepsis, typhoid and scarlet fever, acute lupus erythematosus and gastro-intestinal intoxication. Interesting negative findings were observed in cases of peritonitis, subacute bacterial endocarditis, leukemia and malignancy. Thirty liver sections from normal individuals killed by violence did not show intralobular hepatic edema. None of the positive cases examined revealed early phases of fiber formation. Roessle's hypothesis of the formation of collagen fibers within the serous exudate could not be corroborated in these studies. The present studies failed to reveal any evident injury to the capillary walls or striking necrobiosis of the liver cells due to marked edema. The occurrence of intralobular hepatic edema in a considerable number of cases of cardiac failure was striking. However, it did not occur with such frequency that it might be regarded as merely agonal. Moreover, it was observed in cases presenting no evidence of cardiac failure. In these cases it must be accepted as the result of increased capillary permeability. The differentiation from mechanical edema is difficult. The high incidence of positive findings in malignant nephrosclerosis, uremia, diabetic coma, influenza and Graves' disease deserves further investigation. However, the infrequent occurrence of "serous hepatitis" in most of the infectious-toxic states and the lack of striking parenchymal changes in the positive cases did not permit us to ascribe undue significance to "serous hepatitis" as a pathological entity.

GROWTHS OF PATHOGENIC FUNGI ON MEDIUMS MADE OF HAIR AND SKIN POSSIBLY OF VALUE IN EXPLAINING THE SEQUENCE OF EVENTS IN INFLAMMATION. John W. Williams, Cambridge, Mass.

Abstract. The growth of a large number (34) of pathogenic fungi and of 2 saprophytes has been studied on synthetic as well as natural tissue mediums, such

as hair, skin and horn, for which they have selectivity. Studies were also carried out on mediums prepared from the hydrolytic products of hair and skin. It was found that on hydrolysates of hair, even with considerable dextrose, growth was almost entirely subsurface, while with skin it was partially so, in contradistinction to the predominantly surface growths on the usual peptone mediums. Studies were carried out using cysteine as the source of nitrogen in place of hair, and it was found that the growth was almost identical with that on hair, indicating that with hair the cysteine system was probably responsible for the type of growth. Other amino acids must also be considered since some 8 I have studied show much subsurface growth. They, however, do not possess the active oxidation-reduction system of cysteine-cystine and would be expected to play a more passive rôle in pathological and physiological processes. Freedom from protein and other control may also be necessary for activity.

It is suggested that an oxidation-reduction mechanism involving the cysteine-cystine system plays an important rôle in the subsurface nature of growth and probably in the invasive power of these fungi and the inflammations they produce.

The factors involved in the cysteine-cystine medium and those reported for inflammatory processes, as well as for the natural growth of these fungi *in vivo*, are quite analogous. In regard to autolysis, acidity, reducing action, oxygen tension, glycolysis, osmotic pressure, penetration, and so on, there is marked similarity in inflammatory processes and in the cysteine-cystine mediums when growth takes place. In the latter, the host reaction which, of course, so markedly influences results is absent.

The author does not propose that the cysteine-cystine system is the only mechanism playing a part in invasiveness, inflammation and chronicity, but that in skin and hair it appears to be an important factor. In other tissues it may play only a secondary rôle, or no part whatsoever.

Discussion

(Dr. Norman MacL. Harris, Ottawa.) In view of Dr. Williams' statement regarding the working of the oxidation-reduction process, how does he explain the marked invasiveness of another type of organism — the blastomycetes? I do not recall whether or not he included them in the list shown previously on the screen.

(Dr. Williams, closing.) I have not studied the blastomycetes and do not know about them. They of course may remain chronic a varying length of time. Organisms vary with reference to their ability to overcome barriers and to their selectivity. They probably vary with reference to the oxidation-reduction systems which most influence them. I do not know that I made myself clear but this work is only suggestive. You will note that there was slight surface growth with cysteine-cystine.

In regard to microorganisms, particularly bacteria, I have tried some of them, and my luck has not been especially good.

READ BY TITLE

THE EFFECT OF ANTISYPHILITIC DRUGS ON THE WASSERMANN REACTION. David L. Belding, Boston, Mass.

HYPERTENSION APOPLEXY AND ITS CAUSATION. W. H. Chase, Montreal, Canada.

VIRULENCE AND ANTIGENIC STRUCTURE OF SMOOTH AND ROUGH CULTURES OF *SALMONELLA AERTRYCKE*. G. M. Mackenzie and (by invitation) R. M. Pike, Cooperstown, N. Y.

RENAL CHANGES RESULTING FROM ATTEMPTS TO PRODUCE RENAL SHWARTZMAN REACTION. Alan R. Moritz and Francis Bayless, Cleveland, Ohio.

GLOMERULONEPHRITIS IN AN OPPOSUM, *DIDELPHIS VIRGINIANA*. Herbert L. Ratcliffe, Philadelphia, Pa.

THE MAIN AND ANASTOMOTIC VEINS OF THE ADRENAL. David P. Seecof, Montreal, Canada.

THE VALUE OF DIFFERENTIAL DIAGNOSIS IN TUMORS OF THE BASAL CELL GROUP. Shields Warren and Olive Gates, Boston, Mass.

THE TREATMENT OF ANIMAL TUMORS WITH CYSTEINE HYDROCHLORIDE. C. L. Connor and J. L. Carr, San Francisco.